This is a digital copy of a book that was preserved for generations on library shelves before it was carefully scanned by Google as part of a project to make the world’s books discoverable online.

It has survived long enough for the copyright to expire and the book to enter the public domain. A public domain book is one that was never subject to copyright or whose legal copyright term has expired. Whether a book is in the public domain may vary country to country. Public domain books are our gateways to the past, representing a wealth of history, culture and knowledge that’s often difficult to discover.

Marks, notations and other marginalia present in the original volume will appear in this file - a reminder of this book’s long journey from the publisher to a library and finally to you.

Usage guidelines

Google is proud to partner with libraries to digitize public domain materials and make them widely accessible. Public domain books belong to the public and we are merely their custodians. Nevertheless, this work is expensive, so in order to keep providing this resource, we have taken steps to prevent abuse by commercial parties, including placing technical restrictions on automated querying.

We also ask that you:

+ **Make non-commercial use of the files** We designed Google Book Search for use by individuals, and we request that you use these files for personal, non-commercial purposes.

+ **Refrain from automated querying** Do not send automated queries of any sort to Google’s system: If you are conducting research on machine translation, optical character recognition or other areas where access to a large amount of text is helpful, please contact us. We encourage the use of public domain materials for these purposes and may be able to help.

+ **Maintain attribution** The Google “watermark” you see on each file is essential for informing people about this project and helping them find additional materials through Google Book Search. Please do not remove it.

+ **Keep it legal** Whatever your use, remember that you are responsible for ensuring that what you are doing is legal. Do not assume that just because we believe a book is in the public domain for users in the United States, that the work is also in the public domain for users in other countries. Whether a book is still in copyright varies from country to country, and we can’t offer guidance on whether any specific use of any specific book is allowed. Please do not assume that a book’s appearance in Google Book Search means it can be used in any manner anywhere in the world. Copyright infringement liability can be quite severe.

About Google Book Search

Google’s mission is to organize the world’s information and to make it universally accessible and useful. Google Book Search helps readers discover the world’s books while helping authors and publishers reach new audiences. You can search through the full text of this book on the web at [http://books.google.com/](http://books.google.com/)
THERAPEUSIS OF INTERNAL DISEASES
FORCHHEIMER'S
THERAPEUSIS OF
INTERNAL DISEASES

EDITED BY
FRANK BILLINGS, S.M., M.D.
Professor of Medicine, University of Chicago,
and Rush Medical College, Chicago

AND
ERNEST E. IRONS, M.D., Ph.D.
Assistant Professor of Medicine, Rush
Medical College, Chicago

VOLUME V

NEW YORK AND LONDON
D. APPLETON AND COMPANY
1919
PREFACE

Active immunization by inoculation with bacteria or their products has come into wide use as a means of the prevention and cure of infectious diseases, and when properly and rationally carried out has given results which insure it a permanent place in therapeutics.

The methods of chemotherapy which have proved so successful in the treatment of trypanosomal and other non-bacterial infectious diseases are finding a further application in the combinations of immune sera and chemicals, and a new field of therapeutic possibilities is developing for the treatment of bacterial infections. So also the application of the newer theories of ferments to the parenteral digestion of bacterial protein not only offers an attractive explanation of many of the toxic features common to infectious diseases, but seems destined to modify our ideas of their specific treatment.

We have endeavored first of all to present the more important principles of specific therapy, in relation to their practical application in the treatment of disease, avoiding so far as possible the repetition of definitions and discussions which may be found in the more theoretical treatises. It has seemed well to emphasize the conception of the processes of immunity as a series of chemical reactions, the delicate balance of which may be easily swayed to one side or the other. In the succeeding chapters the diagnostic reactions and the use of sera and vaccines in special diseases are discussed. A corps of writers has been secured especially qualified to furnish responsible opinions upon the subjects to which their names are attached. Therefore, we believe that this volume will have influence in guiding aright those who desire to make proper use of specific immunologic methods of therapy.

FRANK BILLINGS
ERNEST E. IRENS
CONTRIBUTORS TO VOLUME V

JOHN AUER, B.S., M.D.
Associate Member of the Rockefeller Institute, Department of Physiology and Pharmacology, New York
Functional Analysis of Anaphylaxis

FRANK BILLINGS, S.M., M.D.
Professor of Medicine, University of Chicago and Rush Medical College, Chicago
Focal Infection in Relation to Systemic Disease

T. R. BROWN, Ph.D.
Bacteriologist State Board of Health; Director of Hygienic Laboratories, Columbus, Ohio
Pathogenic Fungi—Oidiomycosis including Blastomycosis, and Actinomycosis

RUFUS COLE, B.S., M.D.
Director of the Hospital of the Rockefeller Institute, New York
Pneumococcus Infections

GEORGE FREDERICK DICK, M.S., M.D.
Instructor in Pathology, Rush Medical College, Chicago
Immunological Reactions in Diagnosis

ALPHONSE R. DOCHEZ, A.B., M.D.
Assistant Resident Physician, Hospital of the Rockefeller Institute, New York
Pneumococcus Infections

A. W. ELLIS, M.D.
Assistant Resident Physician, Hospital of the Rockefeller Institute for Medical Research, New York
Specific Treatment of Syphilis of the Central Nervous System

LOUIS HAMMAN, M.D.
Associate in Medicine, Johns Hopkins University and Hospital, Baltimore, Md.
Tuberculin Treatment

N. SPROAT HEANEY, A.B., M.D.
Assistant Professor of Obstetrics and Gynecology, Rush Medical College, Chicago
The Use of Sera and Vaccines in Obstetrics and Gynecology

ERNEST E. IRONS, Ph.D., M.D.
Assistant Professor of Medicine, Rush Medical College, Chicago
Principles of Specific Therapy; Gonococcal Infections; Infections of the Mouth; Anti-
staphylococcus Serum; Antianthrax Serum; Glanders; Rocky Mountain
Spotted Fever; Malta Fever; Hodgkin's Disease

K. K. KOESSLER, M.D.
Assistant Professor of Medicine, Rush Medical College, Chicago
The Specific Treatment of Hay Fever
CONTRIBUTORS TO VOLUME V

W. L. MOSS, M.D.
Associate in Medicine, Johns Hopkins University; Director Research Laboratory, Phipps Tuberculosis Clinic, Johns Hopkins Hospital, Baltimore

Normal Sera and Blood in the Treatment of Anemia and the Hemorrhagic Diseases.

WILLIAM HALLOCK PARK, M.D., A.B., LL.D.
Professor of Bacteriology and Hygiene, New York University and Bellevue Hospital Medical College; Director of Research Laboratories, Department of Health, City of New York; Attending Physician, Willard Parker Hospital, New York

Tetanus

M. MILTON PORTIS, M.D.
Assistant Professor of Medicine, Rush Medical College, Chicago

The Treatment of Graves' Disease Based on Specific Biologic Methods

E. C. ROSENOW, M.D.
Research Member of the Memorial Institute for Infectious Diseases; Associate Professor of Medicine, Rush Medical College, Chicago

The Etiology and Specific Treatment of Rheumatic Fever

FREDERICK F. RUSSELL, Major Medical Corps, U. S. A.
Professor of Bacteriology and Pathology, Army Medical School and Curator, Army Medical Museum, 1907-1913; Professor of Pathology and Bacteriology, George Washington University, Washington, D. C., 1909-1913; Lecturer on Tropical Medicine, Post Graduate Medical School and Hospital, New York, 1914

The Prophylaxis of Typhoid Fever by means of Vaccines

E. MATHER SILL, M.D.
Lecturer in Diseases of Children at the New York Polyclinic Medical School and Hospital; Attending Physician at the New York Polyclinic Hospital; Attending Physician in the Diseases of Children at the Good Samaritan Dispensary, New York

Whooping-Cough

ABRAHAM SOPHIAN, M.D.
Epidemic Cerebrospinal Meningitis

WILLARD J. STONE, B.Sc., M.D.
Attending Physician St. Vincent's Hospital and Flower Hospital, Toledo

Bacterial and Serum Therapy in Typhoid and Paratyphoid Fevers; Bacterial Therapy in Lesions produced by the Bacillus Coli Communis; Snake Venoms and Antisera

RICHARD P. STRONG, M.D.
Professor of Tropical Medicine, Harvard University, Boston

Bacillary Dysentery; Plague; Asiatic Cholera; Leprosy

HOMER F. SWIFT, M.D.
Resident Physician, Hospital of the Rockefeller Institute for Medical Research, New York

Specific Treatment of Syphilis of the Central Nervous System

J. FRANK WAUGH, S.B., M.D.
Assistant in Dermatology, Rush Medical College; Assistant Dermatologist, Presbyterian Hospital, Chicago

Vaccine Therapy in Dermatology with Special Reference to Acne and Furunculosis
CONTRIBUTORS TO VOLUME V

GEORGE H. WEAVER, M.D.
Associate Professor of Pathology, Rush Medical College, Chicago; Attending Physician, Durand Hospital, Memorial Institute for Infectious Diseases, Chicago
Specific Prophylaxis and Therapy in Diphtheria; Specific Remedies in Scarlet Fever; Antistreptococcus Serum

RICHARD WEIL, A.M., M.D.
Assistant Professor of Experimental Therapeutics, Cornell University Medical School; Associate Attending Medical Service of Mt. Sinai Hospital, Assistant Director Cancer Research Service of Cornell University Medical School in the General Memorial Hospital, New York
The Biological Methods of Treatment in Cancer

G. H. WHIPPLE, M.D.
Associate in Pathology, Johns Hopkins Medical School, Baltimore
Normal Sera and Blood in the Treatment of Anemia and the Hemorrhagic Diseases

ANNA WILLIAMS, M.D.
Assistant Director, Bureau of Laboratories, Health Department, New York
Hydrophobia
## CONTENTS

### CHAPTER I

**THE PRINCIPLES OF SPECIFIC THERAPY**

**ERNEST E. IRONS**

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>The Chemical Nature of Immunity</td>
<td>1</td>
</tr>
<tr>
<td>The Development of Immunity</td>
<td>1</td>
</tr>
<tr>
<td>Immunological Reactions as Physico-chemical Processes</td>
<td>3</td>
</tr>
<tr>
<td>Chemical Nature of Antigens</td>
<td>4</td>
</tr>
<tr>
<td>The Relation of the Host to the Invading Organism</td>
<td>6</td>
</tr>
<tr>
<td>The Defense of the Host</td>
<td>6</td>
</tr>
<tr>
<td>The Duration of Passive and Active Immunity</td>
<td>8</td>
</tr>
<tr>
<td>Other Protective Mechanisms of the Body</td>
<td>10</td>
</tr>
<tr>
<td>The Invading Organism</td>
<td>11</td>
</tr>
<tr>
<td>Virulence and Serum-fastness</td>
<td>11</td>
</tr>
<tr>
<td>Specific Chemo-serologic Therapy</td>
<td>12</td>
</tr>
<tr>
<td>The Influence of One Infection on Another</td>
<td>14</td>
</tr>
<tr>
<td>Nonspecific Intoxication as a Cause of the Symptoms of Infectious Disease</td>
<td>15</td>
</tr>
<tr>
<td>Active Immunization—Vaccine Therapy</td>
<td>18</td>
</tr>
<tr>
<td>The Opsonic Curve</td>
<td>19</td>
</tr>
<tr>
<td>The Relation of Opsonins to Recovery from Disease</td>
<td>20</td>
</tr>
<tr>
<td>The Effects of Inoculations on Antibodies Other Than Opsonins</td>
<td>23</td>
</tr>
<tr>
<td>The Clinical Evidence For and Against Active Immunization</td>
<td>25</td>
</tr>
<tr>
<td>Reactions Following the Injection of Bacteria or Their Products</td>
<td>28</td>
</tr>
<tr>
<td>The Neglect of Surgical Procedures</td>
<td>28</td>
</tr>
<tr>
<td>Sensitized Vaccines</td>
<td>28</td>
</tr>
<tr>
<td>Treatment by Intravenous Injections of Foreign Protein</td>
<td>31</td>
</tr>
</tbody>
</table>

**References**

| References                                                 | 33   |

### CHAPTER II

**THE FUNCTIONAL ANALYSIS OF ANAPHYLAXIS**

**JOHN Auer**

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>35</td>
</tr>
<tr>
<td>Historical</td>
<td>36</td>
</tr>
<tr>
<td>Experimental Anaphylaxis</td>
<td>38</td>
</tr>
<tr>
<td>Sensitization</td>
<td>39</td>
</tr>
<tr>
<td>Incubation</td>
<td>43</td>
</tr>
<tr>
<td>Intoxication</td>
<td>44</td>
</tr>
<tr>
<td>The Anaphylactic Reaction</td>
<td>40</td>
</tr>
<tr>
<td>The Experimental Analysis of the Anaphylactic Reaction</td>
<td>51</td>
</tr>
</tbody>
</table>
CONTENTS

Central or Peripheral Causation of the Anaphylactic Reaction ..... 88
Anaphylactic Manifestations in Man ..... 91
Passive Anaphylaxis ..... 96
Antianaphylaxis ..... 98
Prevention of the Anaphylactic Reaction
  Besredka's Methods ..... 102
Criteria of Anaphylaxis ..... 104
Anaphylactoid Phenomena ..... 105
Theories of Anaphylaxis ..... 106
References ..... 108

CHAPTER III

IMMUNOLOGICAL REACTIONS IN DIAGNOSIS

GEORGE F. DICK

Diagnostic Reactions in Vitro ..... 117
  The Agglutination Reaction ..... 119
  Precipitin Reactions ..... 125
  Complement-fixation Reaction ..... 125
  The Immune-ferment Reactions (Abderhalden) ..... 141
  The Miostagmin Reaction ..... 145
  The Epiphanin Reaction ..... 147
  The Opsonins in Diagnosis ..... 148
Diagnostic Reactions Occurring in the Patient ..... 151
  Tuberculosis ..... 151
  Typhoid Fever ..... 158
  Gonococcal Infections ..... 159
References ..... 162

CHAPTER IV

FOCAL INFECTION IN RELATION TO SYSTEMIC DISEASE

FRANK BILLINGS

Site of the Focus ..... 165
Systemic Diseases of Focal Origin ..... 167
  The Relation of Suspected Focus to the Systemic Disease ..... 168
The Pathology of Chronic Systemic Infection of Focal Origin ..... 168
Results of Secondary Foci of Infection ..... 170
Focal Infection and Anaphylaxis ..... 171
Treatment ..... 171
  Prophylaxis ..... 171
  Method of Treatment ..... 173
References ..... 176
CONTENTS

Treatment of Infected Wounds by B. Coli Vaccines 246
References 247

CHAPTER VIII

BACILLARY DYSENTERY

RICHARD P. STRONG

The Bacillus of Dysentery 249
Types of Dysentery Bacillus 249
Differentiation of the Four Types of Dysentery Bacillus 252
The Dysentery Toxin 254
Dysentery Immune Serum 257
Bactericidal Reaction of Dysentery Immune Serum 257
Antitoxic Action of Dysentery Immune Serum 257
Method of Testing Antitoxic Value of Dysentery Serum 258
Instability of Antitoxin 259
Production of Antidysenteric Serum 259
Serum Treatment in Man 260
Dosage 262
Vaccine Treatment in Dysentery 262
References 263

CHAPTER IX

PLAGUE

RICHARD P. STRONG

Vaccine Therapy 266
Serum Therapy 266
Specific Immunizing Properties of the Serum 266
Bactericidal Reaction 266
Anti-infections or Antibacterial and Opsonic Action 268
Result of Treatment in Animals 271
Result of Treatment in Man 272
Treatment in Pneumonic Plague 276
Selection of Serum 277
Method of Testing the Immunizing Value of Serum 277
Varieties of Sera 278
Antitoxic Sera 278
Multivalent Serum 278
References 279

CHAPTER X

ASIATIC CHOLERA

RICHARD P. STRONG

Vaccine Treatment 280
Serum Treatment 280
The Cholera Toxin 280
CONTENTS

Serum Treatment (Continued)
  The Immunizing Properties of Cholera Sera  286
  Effect of Serum in Man  288
  Treatment in Man  288
  Indications for Other Methods of Treatment  292
  Treatment of Enemata of Serum  293
References  294

CHAPTER XI

WHOOPING COUGH (PERTUSSIS)

E. Mather Sill

Introduction  296
Diagnosis  297
Vaccine Treatment  298
  Preparation of Vaccine  304
Vaccine for Prophylaxis  305
Vaccine as a Means of Reducing Infant Mortality  305
Serum Therapy  307
References  308

CHAPTER XII

TUBERCULIN TREATMENT

Louis Hamman

Introduction  309
The Results Obtained with Tuberculin Treatment  310
  Animal Experiments  310
  Clinical Results  310
The Selection of a Tuberculin Preparation  323
  Koch's Original or Old Tuberculin  323
  Denys' Bouillon Filtrate  324
  Jochmann's Albumose-free Tuberculin  324
  Koch's Bacilli-emulsion  324
  Koch's Tuberculin-residue or New Tuberculin  324
  Beraneck's Tuberculin  325
  Von Ruck's Watery Extract  325
  Landmann's Tuberculol  325
Selection of Patients  331
  The General Principles of Tuberculin Treatment  333
  Method of Continuous Minimal Dosage  333
  Method of Increasing Dosage  339
The Preparation of Tuberculin Dilutions and Methods of Administration  344
  The Initial Dose of Tuberculin  346
  Subsequent Doses and Intervals  350
  The Terminal Dose  370
References  372
CONTENTS

CHAPTER XIII

LEPROSY

RICHARD P. STRONG

Micro-organisms Cultivated from Cases of Leprosy .......................... 333
Classification ............................................................................. 333
Uncertainty of the Successful Cultivation of Bacillus Lepros ........................................... 335
Serum Treatment ......................................................................... 337
Vaccine Treatment ....................................................................... 339
Treatment with Nastin ................................................................. 392
Non-specific Vaccine Treatment .................................................... 394
Spontaneous Cure and Improvement in Relation to Treatment ......................... 395
References ................................................................................. 395

CHAPTER XIV

SPECIFIC TREATMENT OF SYPHILIS OF THE CENTRAL NERVOUS SYSTEM

HOMER F. SWIFT AND ARTHUR W. M. ELLIS

General Considerations .................................................................... 397
Salvarsan in Treatment of Syphilis of the Central Nervous System ......................... 408
Relative Value of Salvarsan and Neo-salvarsan ................................................. 409
Local Treatment in Cerebrospinal Syphilis ....................................................... 409
Technique ..................................................................................... 414
Conclusions ............................................................................... 418
References ................................................................................. 419

CHAPTER XV

PATHOGENIC FUNGI—SPOROTRICHOSIS

OIDIOMYCOSIS INCLUDING BLASTOMYCOSIS, AND ACTINOMYCOSIS

T. R. BROWN

Sporotrichosis .................................................................................. 421
Sporotrichum Beurmanni .................................................................. 422
Serum Diagnosis and Immunity ............................................................. 423
Oidionycosia .................................................................................. 424
Oidium Albicans ............................................................................. 424
Blastomycesis ................................................................................ 424
Actinomycosis ................................................................................ 426
Microscopical Appearance of the Parasite .................................................. 425
Gross Appearance .......................................................................... 428
Lesions ......................................................................................... 429
Occurrence of the Parasite in Nature ....................................................... 430
Pathology ...................................................................................... 431
Diagnosis ...................................................................................... 431
Treatment .................................................................................... 431
Madura Foot .................................................................................. 432
Morphology .................................................................................... 432
References ................................................................................... 433
CONTENTS

CHAPTER XVI
SPECIFIC PROPHYLAXIS AND THERAPY IN DIPHTHERIA
G. H. Wood

Introduction .......................................................... 434
Practical Production of Diphtheria Antitoxin .................. 435
Standardization of Antidiphtheric Serum ....................... 437
Distribution, Keeping Qualities and Government Regulation of the
Manufacture of Diphtheria Antitoxin ......................... 439
Specific Prophylaxis Against Infection by Diphtheria Bacilli . 440
Treatment of Diphtheria ........................................... 445
References ........................................................... 450

CHAPTER XVII
TETANUS
WILLIAM H. PARK

The Bacterial Poisons ............................................. 453
The Source of the Infecting Tetanus Bacilli ................. 453
The Means by Which Wound Infection Occurs .............. 454
The Preventive Treatment ....................................... 454
Diagnosis ............................................................ 454
The Paths by Which Tetanus Toxin Reaches the Central Nervous System 455
The Union of Toxin with the Gray Matter of the Brain and Spinal Cord 456
Period Between Absorption of Toxin and Development of Symptoms . 456
Presence of Tetanus Toxin in the Blood ...................... 457
Endotoxins ........................................................... 457
Specific Treatment of Tetanus ................................ 457
The Actual Antitoxic Treatment of a Case of Tetanus ...... 466

CHAPTER XVIII
PNEUMOCOCCUS INFECTION
R. I. COLE AND A. R. DOCHÉZ

General Considerations ......................................... 468
Serum Therapy ..................................................... 473
Vaccinotherapy .................................................... 487
Leukocyte Extract ............................................... 494
Chemotherapy ...................................................... 496
Ulcers Cornææ Serpens .......................................... 502
References ........................................................... 503

CHAPTER XIX
THE ETIOLOGY AND SPECIFIC TREATMENT OF RHEUMATISM
E. C. ROSENOW

General Considerations ......................................... 506
Specific Therapy ................................................... 511
References .......................................................... 512
CONTENTS

CHAPTER XX

EPIDEMIC CEREBROSPINAL MENINGITIS

A. Sophian

Introductory ................................................. 513
Technique of Examining the Cerebrospinal Fluid .......... 515
Pressure of the Cerebrospinal Fluid ....................... 515
Color of the Cerebrospinal Fluid .......................... 516
Fibrin Content ............................................. 516
Chemical Examination of the Cerebrospinal Fluid ....... 518
Bacteriology ............................................... 517
Cytology .................................................... 518

Treatment of Epidemic Meningitis ......................... 521
Classical Method of Performing Lumbar Puncture and Administering Antimeningitis Serum ............................ 524
Specific Treatment of Epidemic Meningitis ............... 526
Serum Treatment of General Bacteremia, Immediately Preceding and During the Course of Meningitis .............. 535
Treatment of Hydrocephalus During the Acute Stage of Meningitis ........................................ 539

Treatment of Subacute and Chronic Meningitis ......... 540
Milder Form of Hydrocephalus .................................. 541
Posterior Basic Meningitis .................................... 542

General Treatment of Meningitis ......................... 547
Treatment of Complications .................................. 548

Analysis of Influences Affecting Prognosis ............... 559

Prophylaxis of Epidemic Meningitis ...................... 563
Quarantine .................................................. 564
Specific Prophylactic Measures ............................. 567

References .................................................. 576

CHAPTER XXI

GONOCOCCAL INFECTIONS

Ernest E. Ibóns

Introduction ................................................. 577
Characteristics of Metastatic Gonococcal Lesions ...... 578
Gonococcal Arthritis ....................................... 578
Articular Fluid ............................................ 581
Iritis ....................................................... 582
Gonococcemia .............................................. 583
Gonococcal Endocarditis .................................. 584
Gonococcal Septicemia .................................... 585
Other Complications of Gonococcemia ..................... 587

Diagnosis .................................................... 590
The Gonococcus ............................................ 591
Methods of Cultivation of the Gonococcus .............. 591
Examination of Blood Exudates and Secretions for the Presence of the Gonococcus ......................... 594
CONTENTS

The Gonococcus (Continued)
Characteristics of the Gonococcus ............................................. 595
Autolysis of the Gonococcus .................................................... 597
Immunity in Gonococcal Infections .......................................... 598
Allergic Reactions ............................................................... 600
Diagnostic Value of Allergic Reactions .................................. 600
Serologic Reactions ............................................................. 602
The Treatment of Gonococcal Infections by Serum and Vaccines .... 606
Treatment of Vaccines .......................................................... 611
Antigonoococcal Serum ......................................................... 618
References ............................................................................. 619

CHAPTER XXII

VACCINE THERAPY IN DERMATOLOGY WITH SPECIAL REFERENCE TO
ACNE AND FURUNCULOSIS

J. Frank Waugh

Introduction ............................................................................... 623
Acne ...................................................................................... 624
Acne Bacillus .......................................................................... 625
Preparation of Vaccine .......................................................... 626
The Reaction After Inoculation .............................................. 627
Treatment .............................................................................. 629
Furunculosis ........................................................................... 631
Preparation and Dose of Vaccine ............................................ 632
Sycosis Vulgaris ....................................................................... 632
Carbunculosis ......................................................................... 633
Eczema .................................................................................. 633
Rosacea .................................................................................. 633
Other Dermatoses .................................................................... 633
References ............................................................................. 634

CHAPTER XXIII

INFECTIONS OF THE MOUTH, INFECTIONS BY FUSIFORM BACILLI AND
SPirochetes, ACUTE ULCEROUS GINGIVITIS, Erysipelas—In-
FECTIONS OF SINUSES—OTITIS MEDIA, SECONDARY
INFECTIONS, MIXED VACCINES

Ernest E. Irons

Infections of the Mouth ............................................................ 635
Infections by Fusiform Bacilli and Spirochetes ......................... 636
Acute Ulcerous Gingivitis ....................................................... 636
Erysipelas ............................................................................... 637
Infections of the Sinuses—Otitis Media .................................... 639
Unwarranted Use of Mixed Vaccine ........................................ 639
References ............................................................................. 641
CONTENTS

CHAPTER XXIV
SPECIFIC REMEDIES IN SCARLET FEVER

G. H. WEAVER

Specific Remedies in Scarlet Fever................. 642
References........................................ 647

CHAPTER XXV
ANTISTREPTOCOCCUS SERUM

G. H. WEAVER

Introduction....................................... 649
References........................................ 653

CHAPTER XXVI
ANTISTAPHYLOCOCCUS SERUM

ERNEST E. IRONS

Antistaphylococcus Serum...................... 654
References........................................ 654

CHAPTER XXVII
ANTIANTHRAX SERUM

ERNEST E. IRONS

Antianthrax Serum................................ 655
References........................................ 656

CHAPTER XXVIII
GLANDERS

ERNEST E. IRONS

Glanders......................................... 657

CHAPTER XXIX
ROCKY MOUNTAIN SPOTTED FEVER

ERNEST E. IRONS

Rocky Mountain Spotted Fever.................. 658
## CONTENTS

### CHAPTER XXX

**MALTA FEVER**  
*Ernest E. Irons*

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>659</td>
</tr>
<tr>
<td>Immunity</td>
<td>660</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>660</td>
</tr>
<tr>
<td>Prophylactic Inoculation</td>
<td>661</td>
</tr>
<tr>
<td>Treatment</td>
<td>661</td>
</tr>
<tr>
<td>Serum</td>
<td>661</td>
</tr>
<tr>
<td>Active Immunization by Killed Cultures</td>
<td>661</td>
</tr>
<tr>
<td>References</td>
<td>661</td>
</tr>
</tbody>
</table>

### CHAPTER XXXI

**HODGKIN'S DISEASE**  
*Ernest E. Irons*

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hodgkin's Disease</td>
<td>662</td>
</tr>
<tr>
<td>References</td>
<td>664</td>
</tr>
</tbody>
</table>

### CHAPTER XXXII

**THE SPECIFIC TREATMENT OF HAY FEVER (POLLEN DISEASE)**  
*Karl K. Koessler*

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Historical Review</td>
<td>665</td>
</tr>
<tr>
<td>Definition</td>
<td>672</td>
</tr>
<tr>
<td>Etiology</td>
<td>672</td>
</tr>
<tr>
<td>Pathogenicity, Individual Susceptibility and Immunity</td>
<td>676</td>
</tr>
<tr>
<td>Specific Treatment</td>
<td>684</td>
</tr>
<tr>
<td>Passive Immunization</td>
<td>684</td>
</tr>
<tr>
<td>Active Immunization</td>
<td>690</td>
</tr>
<tr>
<td>References</td>
<td>698</td>
</tr>
</tbody>
</table>

### CHAPTER XXXIII

**HYDROPHOBIA**  
*Anna Wessels Williams*

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>701</td>
</tr>
<tr>
<td>Definition and Synonyms</td>
<td>701</td>
</tr>
<tr>
<td>History</td>
<td>702</td>
</tr>
<tr>
<td>Geographical Distribution and Prevalence</td>
<td>704</td>
</tr>
<tr>
<td>Animals Affected</td>
<td>705</td>
</tr>
<tr>
<td>Seasonal Prevalence</td>
<td>706</td>
</tr>
<tr>
<td>Pathology</td>
<td>707</td>
</tr>
<tr>
<td>Gross Pathology</td>
<td>707</td>
</tr>
<tr>
<td>Histologic Pathology</td>
<td>707</td>
</tr>
</tbody>
</table>
CONTENTS

Etiology
   Negri Bodies
Incubation
Symptoms
   Symptoms in Man
Diagnosis
Treatment
   Specific Treatment
References

CHAPTER XXXIV

SNAKE VENOMS AND ANTISERA

WILLARD J. STONE

Historical
The Toxic Constituents of Venoms
Cobra Venom Solutions in the Diagnosis of Syphilis and Tuberculosis
Antisera
References

CHAPTER XXXV

THE BIOLOGICAL METHODS OF TREATMENT IN CANCER

RICHARD WEIL

Introductory
Experimental Data
   Passive Immunization
   Active Immunization
   The Relationship Between Clinical and Experimental Data
The Criteria of Therapeutic Effectiveness in Human Cancer
Clinical Data
   Results of Serum Therapy in Human Beings
   Vaccine Therapy
Summary
   Other Biological Methods
References

CHAPTER XXXVI

THE TREATMENT OF GRAVES' DISEASE BASED ON SPECIFIC BIOLOGIC METHODS

M. MILTON PORTIS

Introduction
General Treatment
Treatment with Sera

PAGE
708
710
712
713
714
715
717
719
741
743
743
745
746
748
750
751
751
762
769
773
773
775
782
783
784
789
792
793
CONTENTS

CHAPTER XXXVII
NORMAL SERA AND BLOOD IN THE TREATMENT OF ANEMIA AND THE HEMORRHAGIC DISEASES
G. H. WHIPPLE AND W. L. MOSS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>The Mechanism of Coagulation of the Blood (G. H. Whipple)</td>
<td>795</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>796</td>
</tr>
<tr>
<td>Calcium</td>
<td>796</td>
</tr>
<tr>
<td>Prothrombin</td>
<td>796</td>
</tr>
<tr>
<td>Antithrombin</td>
<td>797</td>
</tr>
<tr>
<td>Other Factors</td>
<td>797</td>
</tr>
<tr>
<td>Classification and Treatment of the Anemias and Hemorrhagic Diseases (W. L. Moess)</td>
<td>798</td>
</tr>
<tr>
<td>Anemia</td>
<td>799</td>
</tr>
<tr>
<td>Hemorrhagic Diseases</td>
<td>801</td>
</tr>
<tr>
<td>Diseases With Which Hemorrhage May Be Associated</td>
<td>801</td>
</tr>
<tr>
<td>Methods of Treatment</td>
<td>802</td>
</tr>
<tr>
<td>Application of the Methods of Treatment</td>
<td>808</td>
</tr>
<tr>
<td>References</td>
<td>817</td>
</tr>
</tbody>
</table>

CHAPTER XXXVIII
THE USE OF SERA AND VACCINES IN OBSTETRICS AND GYNECOLOGY
N. SPROAT HEANEY

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>819</td>
</tr>
<tr>
<td>Serum, Desfibrinated Blood, and Whole Blood in the Treatment of Hemorrhagic Diseases</td>
<td>820</td>
</tr>
<tr>
<td>Hemorrhagic Disease of the Newborn</td>
<td>822</td>
</tr>
<tr>
<td>Serum in the Treatment of Uterine Bleeding</td>
<td>823</td>
</tr>
<tr>
<td>Human Serum in the Induction of Labor</td>
<td>824</td>
</tr>
<tr>
<td>Normal Serum in the Treatment of the Intoxications of Pregnancy</td>
<td>824</td>
</tr>
<tr>
<td>The Use of Antistreptococcus Serum</td>
<td>826</td>
</tr>
<tr>
<td>The Treatment of Puerperal Infection by Vaccines</td>
<td>827</td>
</tr>
<tr>
<td>Vaccine Treatment of Gonorrheal Infections of the Female Genitalia</td>
<td>829</td>
</tr>
<tr>
<td>Vulvovaginitis</td>
<td>833</td>
</tr>
<tr>
<td>Lactic Acid Bacilli in Vaginitis</td>
<td>833</td>
</tr>
<tr>
<td>Treatment of Female Genital Tuberculosis by the Use of Tuberculin</td>
<td>834</td>
</tr>
<tr>
<td>References</td>
<td>834</td>
</tr>
<tr>
<td>INDEX</td>
<td>839</td>
</tr>
</tbody>
</table>
# LIST OF ILLUSTRATIONS

## THE FUNCTIONAL ANALYSIS OF ANAPHYLAXIS

**JOHN AVER**

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Volume Changes of the Lung in a Guinea-pig During Acute Anaphylaxis</td>
<td>53</td>
</tr>
<tr>
<td>2.</td>
<td>Anaphylactic Lung in the Guinea-pig, With and Without Atropin</td>
<td>55</td>
</tr>
<tr>
<td>3.</td>
<td>Electrocardiogram Before Injection</td>
<td>65</td>
</tr>
<tr>
<td>4.</td>
<td>Dissociation—Onset of Partial Auriculo-ventricular Dissociation</td>
<td>66</td>
</tr>
<tr>
<td>5.</td>
<td>Partial Auriculo-ventricular Dissociation of a Higher Degree</td>
<td>66</td>
</tr>
<tr>
<td>6.</td>
<td>Partial Auriculo-ventricular Dissociation Due to Anaphylaxis</td>
<td>67</td>
</tr>
<tr>
<td>7.</td>
<td>Anaphylactic Drop of Blood Pressure in Dog</td>
<td>71</td>
</tr>
</tbody>
</table>

## IMMUNOLOGICAL REACTIONS IN DIAGNOSIS

**GEORGE F. DICK**

<table>
<thead>
<tr>
<th>Number</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Aspirator for Obtaining Blood</td>
<td>118</td>
</tr>
<tr>
<td>2.</td>
<td>Arrangement of Test Tubes for the Wassermann Reaction</td>
<td>132</td>
</tr>
</tbody>
</table>

## THE PROPHYLAXIS OF TYPHOID FEVER BY MEANS OF VACCINES

**FREDERICK F. RUSSELL**

<table>
<thead>
<tr>
<th>Number</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Chart Showing Admission Rates for Typhoid Fever, United States (Enlisted Men)</td>
<td>193</td>
</tr>
<tr>
<td>2.</td>
<td>Chart Showing Death Rates for Typhoid Fever, United States (Enlisted Men)</td>
<td>194</td>
</tr>
<tr>
<td>3.</td>
<td>Chart Showing Non-effective Rates for Typhoid Fever, United States (Enlisted Men)</td>
<td>194</td>
</tr>
</tbody>
</table>

## WHOOPING-COUGH (PERTUSSIS)

**E. MATHER SILL**

<table>
<thead>
<tr>
<th>Number</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Bordet's Pertussis Bacillus Pure Culture</td>
<td>297</td>
</tr>
<tr>
<td>2.</td>
<td>Epithelial Cells of Trachea and Bronchus of a Child Under Normal Conditions and in Whooping-cough</td>
<td>299</td>
</tr>
</tbody>
</table>
LIST OF ILLUSTRATIONS

TUBERCULIN TREATMENT

LOUIS HAMMAN

<table>
<thead>
<tr>
<th>FIGURE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Chart Showing Rise of Temperature Due to Tonsilitis; Not to Tuberculin Reaction</td>
<td>356</td>
</tr>
<tr>
<td>2. Chart Showing Rise of Temperature Due to Secondary Syphilis and Not to Tuberculin Reaction</td>
<td>356</td>
</tr>
</tbody>
</table>

TETANUS

WILLIAM H. PARK

<table>
<thead>
<tr>
<th>FIGURE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Chart Showing Extent and Rapidity of Absorption of 10,000 Units of Antitoxin Given Subcutaneously</td>
<td>462</td>
</tr>
<tr>
<td>2. Chart Showing the Antitoxic Power of Human Blood After an Intravenous Injection of 10,000 Antitoxic Units</td>
<td>462</td>
</tr>
</tbody>
</table>

MALTA FEVER

ERNEST E. IRONS

<table>
<thead>
<tr>
<th>FIGURE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Curves of Temperature and Titre of Serum with Respect to Agglutinins</td>
<td>660</td>
</tr>
</tbody>
</table>

HYDROPHOBIA

ANNA WESSELS WILLIAMS

<table>
<thead>
<tr>
<th>FIGURE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Inoculation of Rabbit for Production of “Fixed Virus”</td>
<td>721</td>
</tr>
<tr>
<td>2. First Step in Removing Fixed Virus Spinal Cord from Rabbit</td>
<td>722</td>
</tr>
<tr>
<td>3. Second Step in Removing Fixed Virus Cord from Rabbit</td>
<td>723</td>
</tr>
<tr>
<td>4. One Corner of Constant Temperature Room, Showing Drying Bottle, Containing Fixed Virus Cords, Being Prepared for Vaccine</td>
<td>724</td>
</tr>
</tbody>
</table>
THE PRINCIPLES OF SPECIFIC THERAPY

Ernest E. Irons

THE CHEMICAL NATURE OF IMMUNITY

Whenever the physiological processes of the body are interfered with, whether by the invasion of micro-organisms, or from some other physical or chemical cause, there results a series of physical and chemical changes not present before, which we call disease. These new changes are the outcome of chemical and physical rearrangements which must follow on the introduction of new substances into the system of substances previously in physiological equilibrium. The natural tendency of disturbed physiological processes is to return to normal, and so in the vast majority of cases interference with the physico-chemical processes of the body results in only a temporary disturbance of normal function. The return to normal function may, however, be delayed, and clinical experience and laboratory experiment teach that under some circumstances the return to normal function may be hastened by the giving of certain drugs, which act by rendering the cause of the disturbance inert, or by stimulating the physiologic processes to more rapid action.

THE DEVELOPMENT OF IMMUNITY

The problem of recovery from infectious disease, or the development of immunity, may be conceived of as involving a series of readjustments of disturbed physico-chemical processes, quite similar to those in non-infectious forms of disease. We have to deal, however, with two antagonistic groups of processes, those of the invading organism and those
of the host. The outcome of the struggle between invader and host will depend on the resultant of these extremely complex and interrelated forces. Their adjustment is one of great delicacy, and seemingly unimportant factors may serve to sway the balance to one side or the other. The invading organism may exert its unfavorable action on the host by means of a soluble toxin in the one case, by toxic substances set free by its death in another, or perhaps by mechanically causing obstructions in vessels and thus interfering with the function of vital organs.

The Specific Defense of the Host.—The host, on his side, protects himself by the elaboration of antitoxin to neutralize toxin, of substances which act injuriously on the invader, bacteriolyisins, or by the engulfing and digestion of bacteria by migratory cells of the body. As in the non-infectious disturbances of physiological equilibrium, the reactions of the body tend to readjustment by the elimination of abnormal substances; expressed clinically, infections tend to result in immunity. The formation of defensive substances is specific for each organism; the antitoxin formed to defend the body against diphtheria toxin will neutralize only diphtheria toxin; tetanus antitoxin will neutralize only tetanus toxin. Blood which is bactericidal for typhoid bacilli may have no effect on plague bacilli. The specificness of the defense complicates the study of immunity, but need not preclude the conception of it as a chemical process; as will be seen later, the specificity of antibodies argues for an adjustment of chemical structure of a particularly fine nature, not recognizable by the ordinary methods of clinical examination at our command.

Specific Therapy.—Specific therapy aims to assist the natural forces of the body in their struggle with the invading organism, either by supplying substances which shall neutralize the poisons of the invader (antitoxin), or by stimulating cells of the body not engaged in the struggle, to reinforce by the formation of various antibodies the efforts of those cells already involved in a local infection. Specific therapy also is concerned with the application of certain drugs, either in their natural forms or combined in organic compounds, which shall act injuriously on the invading organism, at the same time leaving the cells of the host unharmed. Mercury and quinin are commonly cited examples of the former class, salvarsan, and other similar combinations of arsenic, of the latter. Thus far attempts at specific chemotherapy have been successful for the most part in the treatment of non-bacterial infections, such as those due to trypanosomes or spirochetes. There are, however, many indications that the principles of chemotherapy in this narrower sense may find a much wider application, extending even to the treatment of diseases due to the more commonly recognized organisms, such as the pneumococcus, streptococcus, and tubercle bacillus. The more recent studies in vital staining, in which various dyestuffs are found to combine with bacteria, giving
reactions dependent on the chemical constitution of the cell substance, are suggestive of the possibilities of chemotherapy.

**Immunological Reactions as Physico-Chemical Processes**

Studies of the disturbances of normal equilibrium which take place in the tissues and fluids of the body in response to the introduction of foreign substances of bacterial or other protein nature have resulted in the discovery of an immense number of facts, and the demonstration of a number of properties of normal and immune serum which constitute the data of immunology. The further discovery of new facts and reactions has been facilitated by the grouping of facts already known into systems and theories, such for example as the side chain theory of Ehrlich and the ferment theory of Abderhalden. In the explanation of immunological processes and reactions, chemical conceptions have occupied an increasingly prominent place, and it has become evident that in studies on antibodies we are dealing with the same classes of chemical substances, colloids, crystalloids, with which the physiologic chemist experiments, and, further, that the resultant reactions are governed by the same physico-chemical laws of osmosis, electrolytic dissociation, mass action, surface tension, temperature, and concentration. The extreme delicacy and high degree of specificity of biological reactions place them in a position of isolation from other groups of chemical reactions, but the gulf which years ago appeared too wide and deep ever to be bridged is now spanned by many connecting theories supported by well-established facts.

If, for example, we study the agglutination of bacteria by immune serum, which action we attribute to the presence of antibodies, called from their action agglutinins, we are at once met with the fact that this process of agglutination requires the presence of electrolytes, that its rate is influenced by temperature, concentration of bacteria and of serum. We further find that agglutination specific for one group of bacteria is a property not entirely unique to immune serum. A similar specificity of agglutination may be obtained with dilute mineral acids, and the specificity may be varied for different groups of bacteria by varying the concentration of the acid solutions. Other similar examples present themselves in the study of precipitins.

The recently developed colloidal gold reaction of Lange is another familiar example of the relation of physico-chemical conditions to specific reactions of albumins.

Zsizmondy (15) found that certain albuminous bodies when brought in contact with a solution of colloidal gold in the presence of an electrolyte would in certain concentrations cause a clumping together of the small colloidal particles, with a resulting change in color of the solution and later precipitation of the particles of gold. This precipitation was
prevented if the concentration of the albumin was increased. The degree of concentration at which precipitation ceased and protection began was different for each albumin. Lange applied these facts to the examination of cerebrospinal fluids and found that, by making a suitable series of dilutions of the fluid, color reactions may be obtained with the colloidal gold, apparently specific for the several diseases known by other methods to produce changes in the cerebrospinal fluid. Thus the concentrations of the fluid at which color changes or precipitation occurs in fluid from cases of tubercle differ from those giving reactions when fluid from supplicative meningitis is used. Quite apart from the question of the reliability of the test as a diagnostic procedure, the phenomenon affords a striking demonstration of the chemical nature of specificity. As might be expected, any deviation, however slight, from the prescribed method of preparation of reagents interferes with the physico-chemical conditions of the reaction and results in discordant and non-specific reactions.

Chemical Nature of Antigens

The structural and physical relations of the substances which have antigenic properties (i.e., are able to stimulate the production of specific antibodies when introduced into the living animal) are of interest, not only from the point of scientific research, but by reason of the direct bearing of the question on problems of therapy. None of the substances, the exact chemical structure of which is known, possesses true antigenic properties, although it is possible that certain poisons for example, whose chemical structure is known, may combine with albumin by a process of adsorption to form substances having specific antigenic powers, as evidenced by the formation of antibodies for these poisons or their combinations (E. P. Pick, 9). In general the presence of protein in a substance is essential to antigenic power. The number of the antibodies produced by antigens probably varies with the size of the antigen molecule. Thus diphtheria toxin produces only antitoxin, and may be regarded as monovalent in distinction from polyvalent albumins which give rise to a number of immune bodies, such as agglutinins, precipitins, and lysins in the same serum. The valence of an antigen appears to be closely associated with the size of its molecule, as shown by the relatively more rapid diffusion of monovalent antigens, such as diphtheria toxin or cobra toxin through osmotic membranes, as compared with polyvalent antigens. The alteration of albumin by splitting it into simpler substances changes its antigenic qualities and eventually destroys them entirely.

The reactions of antigens and their antibodies present in many respects a close analogy with the reactions of other colloidal substances. Both are influenced by physico-chemical conditions, such as the degree of
THE CHEMICAL NATURE OF IMMUNITY

acidity or alkalinity of the menstruum, relative solubilities and concentration, electrical charge, temperature, and surface tension.

Landsteiner has divided the reactions of immunity into two groups, the first of which comprises those involving the simple union of two colloids, as exemplified in agglutination, precipitation, and the neutralization of toxin by antitoxin; the second of which includes those reactions involving the solution or destruction of cell membranes through the action of colloids (antibodies) on the lipid-albumin combination of the membranes. Examples of this latter class of reactions are the phenomena of hemolysis and bacteriolysis.

Further, the antigenic qualities of an albumin may be modified by physico-chemical means, such as the application of heat, or exposure to various chemicals, such as acids, chloroform, toluol, or metals, as iron, lead, and mercury. This treatment need not result in the complete alteration of the albumin molecule, but may affect only certain groups. If rabbit serum is treated with concentrated nitric acid and the resulting nitro-albumin (xanthoprotein) used for immunizing the rabbit, an immune serum is obtained which will precipitate not only the rabbit nitro-protein, but also nitro-proteins prepared from other foreign albumins. If foreign nitro-protein is used for immunization, the resulting immune serum will precipitate the corresponding and other foreign nitro-protein similarly to the serum obtained from the homologous serum antigen. Both sera show relatively little specific precipitating power with respect to the corresponding albumins from which the nitro-proteins were obtained (E. P. Pick, 10). This loss of antigenic power with respect to the specific albumins, and the gain of antigenic power for nitro-proteins in general, are regarded as additional evidence that the quality of specificity resides in the slight variations in side groups attached to the central albumin molecule, and that by substituting one group for another the specific antigenic qualities of an albumin may be modified.

The possibility of altering specifically the antigenic qualities of a protein furnishes another means of approach to the problem of immunization against disease. It has long been known that if erythrocytes of a given species are saturated with a corresponding hemolytic immune serum they lose the power of stimulating the production of hemolytic antibodies when injected into a foreign species. This loss of antigenic power has been explained on the supposition that the specific groups of the erythrocytes have been occupied by antibodies of the immune serum, and are no longer able to unite with receptors of the cells of the animal into which they are introduced and hence do not stimulate the further formation of these antibodies.

Bacteria treated with corresponding immune sera appear to be less toxic for animals than untreated bacteria, and some observers have noted a decrease in the antigenic power of the treated bacteria for the produc-
tion of agglutinins and precipitins, with an increase in the production of bactericidal substances. (Cf. Sensitized Vaccines.)

THE RELATION OF THE HOST TO THE INVADING ORGANISM

The formulation of a rational treatment of an infectious disease requires in the first place a knowledge of the nature of the infecting organism. Gradually the lines of differentiation of the infections have been more clearly defined, so that the entity of many, such as diphtheria, typhoid fever, epidemic meningitis, cholera, plague, has been established, and the specific organism causing them discovered. Other diseases, such as the acute exanthemata, are fairly well defined clinically, but we know but little of their etiology beyond the presumptive evidence that they are caused by some form of living organism.

The discovery of the causative organism of a disease does not, however, solve the problem of its specific therapy, and is only the first step toward the determination of the sequence and relation of chemical processes and reactions by which the symptoms of the disease are brought about, and by which the disturbed physiological equilibrium is returned to normal.

These processes concern those of the offense of the invading organism and those of the defense and offense of the host. Recently more attention has been given to the changes in the invading organism by which it may increase its defense against the counter-attack of the host.

THE DEFENSE OF THE HOST

Antibodies.—Among the most readily demonstrable changes which occur in an animal in response to invasion by a micro-organism are the new properties acquired by the blood serum, which are indicated by the names, antitoxin, agglutinin, precipitin, bacteriolysin, opsonin, descriptive of the nature of their several actions. Much has been learned of the nature of these antibodies to bacteria and their products by a study of the antibodies produced in response to the inoculation of other foreign cells and proteins, by which hemolysins, cytotoxins, or precipitins are formed. It is important to bear in mind that we recognize antibodies in sera to a large extent by the physical changes which they produce in cells or fluids to which they are added; that, so far as we know, the number of classes of recognized antibodies is limited only by the number of methods which have been devised for their demonstration, and that, while they exhibit a degree of specific action not attained by other chemical processes, this specificity does not argue against the basic physico-chemical action of antibodies, but rather for a particularly fine adjustment of chemical structure. The introduction of an antigen is the most efficient, and usu-
ally the only, method we possess of producing other substances (antibodies) which shall meet exactly the physico-chemical conditions necessary for union with the antigen.

**Ehrlich's Theory.**—From time to time theories have been evolved based on generalizations from groups of facts observed in relation to the changes produced in animals by the introduction of foreign protein. Perhaps the theory most useful in promoting investigation in immunity has been that developed by Ehrlich. This receptor or lateral chain theory was first formulated to explain the assimilation of food by cells and later was expanded to cover the production and action and standardization of diphtheria antitoxicin. The theory has been widely employed in the classification and explanation of other reactions of immunity, so that the terminology of the subject of immunity is largely that of the side-chain theory. The theory, already familiar to all, is based on the supposed analogy between the products of the cell and complex chemical substances, such as those containing the benzin ring, the special chemical properties of which are determined by the attached side groups or radicals. It assumes that the cell possesses certain groups or receptors capable of combining with foreign substances, and that, when these receptors are occupied by the combining or haptophore groups of the foreign substance (antigen), new receptors are formed by the cell. Following Weigert's law of over-compensation in regeneration, an excess of receptors is formed and cast off into the blood. These cast-off receptors constitute the antibodies. The great excess of free receptors produced in response to some types of immunization, as, for instance, in antitoxicin formation, has been explained by some on the theory of stimulation of the cells by the toxin, rather than by the more limited action of Weigert's principle of over-compensation.

Ehrlich has divided antibodies into groups according to their mode of action. In the first order he placed the antitoxins, which possess one combining group—that for the toxin molecule. In the second order are those antibodies which possess a combining group for the antigen and an "ergophoric" group, by which the antibody exerts its characteristic action on the antigen, e. g., agglutinins, precipitins. The third order of antibodies includes those which possess two combining groups, one for the antigen, and one for a third substance complement, which is the active agent in promoting changes in the antigen, e. g., lysins, bacteriolysins. Antibodies of this class thus serve to bring together, or make possible, a reaction between antigen and the third substance (complement), and hence have been termed amboceptors.

Objections have been raised to the lateral chain theory of Ehrlich on the ground that it presupposes an unnecessarily complicated system, and that the terminology is cumbersome. If, however, each term of the theory is conceived of as descriptive of a combination of physical conditions and chemical structure, which, when reproduced under constant conditions,
may be depended on to react in a constant manner, specificity is seen to be as much a chemical property as the reactions of precipitation of metals and salts in inorganic chemistry; and the complicated terminology is merely an expression of the exacting conditions under which the reactions of immunity take place. The terminology has been unnecessarily complicated by the introduction of several terms for the same substance or idea. Thus, for instance, immune body, amboceptor, preparator, fixateur, substance sensibilisatrice, have been used by various workers to designate the same property in immune serum.

Mass Action in Passive Immunity

The conceptions of Ehrlich in regard to certain of the fundamental facts of the reactions of antigen and antibody have not been accepted in their entirety by other workers. For example, Ehrlich held that the union of toxin and antitoxin is an irreversible reaction, while Arrhenius and Madsen have contended that the process is governed by laws of mass action, and that accordingly in a theoretically neutral mixture of toxin and antitoxin there is always a small amount of free toxin present. In the presence of an excess of antitoxin the amount of free toxin grows smaller. Experiments with osmotic membranes indicate that the latter view is more nearly correct, and that diphtheria toxin may be separated from antitoxin with which it has united. The process of separation is a very slow one, but the experimental results show that the union is not entirely permanent.

The Importance of Large Doses of Antitoxin.—The application of the laws of mass action to the union of toxin and antitoxin is of practical importance in the treatment of such diseases as diphtheria and tetanus. When the patient comes under treatment he has more or less free toxin circulating in the blood, and it is essential that as much as possible of this toxin shall be immediately neutralized and prevented from becoming fixed in vulnerable tissue cells. To accomplish this, large initial doses of antitoxin will be more effective than smaller doses, even though the latter might be just sufficient to neutralize all the toxin present. In some urgent cases of diphtheria and in all cases of tetanus, to be of full value in saving life, the antitoxin must reach the blood more rapidly than is possible by the slow absorption from subcutaneous tissues which only reaches the maximum after 48 to 72 hours, and the intravenous injection offers a rapid means to this end.

The Duration of Passive and Active Immunity

The relatively short duration of passive immunity acquired by the introduction of an immune serum, as compared with the more lasting
active immunity obtained by the direct inoculation of toxins or other antigens, is generally recognized, but the importance of distinguishing between the two types is so great that a reference to the subject seems warranted.

In general, when diphtheria antitoxin or tetanus antitoxin is given subcutaneously, the amount of antitoxin in the blood increases gradually, reaching its height about 48 to 72 hours after the injection, and then decreases slowly until at the end of ten days or two weeks very little is left in the blood. If the antitoxin is given intravenously the concentration of antitoxin in the blood reaches the maximum much earlier, and then slowly decreases at about the same rate as when given subcutaneously. Clinically, protection ceases after the third week.

Antibodies derived from homologous sera disappear more slowly than those from alien sera. Thus in experiments recently reported by Lüdke and Orndschiew (8) the agglutination titre of the blood of rabbits for dysentery bacilli rises rapidly after the subcutaneous or intravenous injection of specific immune goat serum of high agglutinating titre, and then falls rapidly, and at the end of eight days reaches the level of agglutinative power present before the injection of the serum. If, however, immune rabbit serum is used for the injection of rabbits the titre rises rapidly as before, but falls more slowly, reaching its normal level only after 20 to 30 days. Similar results were obtained in man, using goat and human sera agglutinative for typhoid bacilli. Agglutinins derived from goat serum disappeared usually by the sixth day, while agglutinins derived from highly immune human serum were still demonstrable on the fourteenth day.

These experiments were made with serum containing no appreciable trace of the specific antigen used in their production. Where immune goat serum containing a small amount of antigen was injected into rabbits or man the agglutinins remained high for longer periods and were still present at the last examinations made, 30 days after injection. These results conform to those of earlier workers. Theobald Smith (11) conducted a series of experiments based on the fact that the offspring of female guinea-pigs immunized to diphtheria toxin inherit a demonstrable antitoxic immunity. He showed that mixtures of antitoxin and toxin in which the antitoxin is present in great excess produce relatively little or no lasting immunity, but that as the proportion of toxin increases the immunity becomes more lasting, and that by the injection of suitable toxin-antitoxin mixtures which have no harmful effects, either immediate or remote, an active immunity lasting several years can be produced in guinea-pigs. This combination of passive and active immunization may find an important application in the prophylaxis of diphtheria (q. v.).

In general, the duration of passive immunity is limited to days or weeks. Active immunity, on the other hand, may last for months, years,
or even for life. Much seems to depend on the degree of thoroughness with which the body is sensitized, recovery from a mild attack of the disease being the most efficient method of sensitization. Some diseases such as pneumonia, erysipelas, and gonorrhea apparently confer an immunity which persists for a relatively short period. However, it may be that in pneumonia at least, the immunity produced is referable only to the specific strain of pneumococcus concerned in the attack, and that subsequent attacks may be caused by unrelated strains.

**OTHER PROTECTIVE MECHANISMS OF THE BODY**

The theories of immunity most extensively applied thus far in researches into the mechanism of immunity have been those related to changes in the serum, antibodies and ferments, believed to be derived from fixed or mobile cells in response to the stimulation of the infecting organism, and those which have to do with increase of phagocytic activity of leukocytes and other cells, acting alone or by the assistance of serum containing opsonin.

In addition to these, a number of other derivatives of body cells have been found to have definite bactericidal action. Leukocytes yield substances which are thermostable and bactericidal. Hiss and Zinsser (7) found that extracts prepared from the leukocytes obtained from rabbits following the intrapleural aleuronat injections were able to modify the course of pneumococcus, staphylococcus, meningococcus, typhoid, and cholera infections in animals, and that in many cases the animals were saved from an otherwise fatal dose. These authors believed also that lobar pneumonia, erysipelas, and cerebrospinal meningitis in man could be favorably influenced by the inoculation of such extracts.

Opie found that otherwise fatal experimental intrapleural tuberculous infections in dogs could be made to heal by the introduction of living dog leukocytes, and Manwaring noted a similar protective influence of leukocytes in experimental tuberculous meningitis of dogs. It is believed that certain of these non-specific protective substances may act as ferments; other substances such as the soaps of fatty acids may act indirectly on the invading organism by modifying its chemical relations to other protective substances or cells.

The production of the toxic phenomena of disease by the non-specific derivatives of the proteolysis of bacterial cells in diseases such as typhoid fever may be cited as an instance of how reactions which are primarily protective may become antagonistic to the life of the host (cf. page 16). Kolačzek and others have urged further that the general symptoms such as fever, which accompany local abscess formation, are referable to the toxic action not only of the products of bacterial proteolysis, but also of proteolysis of dead tissues of the body, whose solution has been brought
about by leukocytic ferments present in the abscess cavity. On the basis of the observation that albuminous fluids such as those of ascites or pleural effusion or blood serum itself are able to stop this proteolytic action in vitro, Kolaczek has suggested the treatment of acute phlegmons and abscesses by the injection of such albuminous fluids. The favorable results which have been observed from this treatment in the decrease of symptoms of general intoxication and local destruction of tissue may be due in part to the so-called "antiferment" action of the serum, but there must also be taken into account the effect of relief of tension in the abscess which follows the puncture and evacuation of the contents of the abscess, as well as the possible action of fresh leukocytes, opsonin, and amboceptors, introduced in the serum.

THE INVADING ORGANISM

In general we may designate a micro-organism infectious if it is able to multiply and produce symptoms of disease in the animal body. In order to produce disease it must enter the body, and in doing so must overcome obstacles, some mechanical, others functional, of the cells and fluids of the host. The rapidity and extent of the invasion depend, in part, on the readiness with which the organism assumes a parasitic existence in the host, the site of entrance into the body, the size of the initial dose, and the resistance of the invader to the attack of the defensive forces of the host. Particular races or strains of an organism may acquire special qualities by which they are better able to gain entrance to and maintain themselves in a special organ of the host, "organ affinity"; or during their residence in the host they may be so modified that they are no longer susceptible to the previous lethal action of body fluids, "serum-fastness"; or they may come to occupy portions of the body relatively inaccessible to the defensive substances of the body.

The recognition of these latter factors is of great importance in devising and applying therapeutic measures. Thus antimeningococcic serum is unable to bring about the cure of epidemic meningitis if injected subcutaneously, but if introduced into the subarachnoid space by lumbar puncture has a prompt effect in promoting the phagocytosis and solution of meningococci, and finally accomplishes the cure of the disease.

VIRULENCE AND SERUM-FASTNESS

"Fastness" of organisms by which they become relatively insusceptible to the destructive action of immune sera and phagocytes, has been partially explained in various ways. Some degree of serum-fastness is probably a component of the initial virulence by which an organism gains a foothold.
in the body; the presence of a capsule or relative increase in thickness of the ectoplasm are frequently noted in virulent strains and in those recently isolated from lesions in animals, and have been regarded as the means by which some organisms increase their resistance. Virulent bacteria differ from the avirulent by the presence within or about them of substances which may act either as direct physico-chemical repellants to the leukocytes (negative chemotaxis), or may interfere with the specific opsonic action of serum, and so prevent phagocytosis. Thus Rosenow, in a study of virulent pneumococci, attributed their resistance to phagocytosis to the presence of a substance which he termed "virulin"; after the extraction of this substance, previously resistant pneumococci became phagocytatable, and avirulent readily phagocytatable pneumococci when treated with "virulin" became resistant to phagocytosis. The action of the aggressins of Bail (derived from the peritoneal exudate of animals inoculated with living bacteria) in increasing the power of a bacterial suspension to produce fatal infection in a second animal has been thought by some to be due to endotoxins and other bacterial products, which reinforce the toxic action of the inoculated bacteria; by others their action has been regarded as directed against the leukocytes.

Fastness may also be exhibited by organisms with respect to immune sera which are known to exercise bactericidal action. Flexner (4) has noted that in certain cases of epidemic meningitis which fail to respond to treatment with antimeningococcic serum there are indications that the organisms belong to strains relatively more resistant to the action of the serum. Serum-fast strains may also develop in the course of an infection, and the fatal relapses, following the initial improvement under serum, may be caused by strains which have become more resistant to serum action.

The well-known experiments of Ehrlich on infections by trypanosomes have demonstrated that acquired fastness is an important factor in chemotherapy and that exposure of organisms to the action of chemical substances of known formula may result in the appearance of strains with increased resistance to the special substance used.

The modifications exhibited by bacteria during their sojourn in the host are, however, no more striking than the changes in growth, resistance, and toxin formation in the culture tube in response to alterations in physical and chemical environment, but the acquisition of these new qualities within the host and the development of more resistant sub-strains during the course of a chronic infection further complicate the difficult problem of therapy.

**SPECIFIC CHEMO-SEROLOGIC THERAPY**

The knowledge of the mechanism by which each micro-organism protects itself against its host makes it possible to devise methods of over-
coming this resistance, and already improvements in practical therapy have been made with this principle as a guide. Polyvalent antisera in which known resistant strains are included in the group of bacterial strains used in the production of the serum have been suggested to overcome the serum-fast strains of meningococci. Strains of trypanosomes, fast with respect to one chemical, have been overcome by the use of a second closely allied chemical.

The experiments on pneumococci described by Flexner (loc. cit.) illustrate at once the value of the conception of immunity as a problem of immuno-chemistry, and the importance of the adjustment of chemical relations to meet the known biologic peculiarities of the organism. The essential data of the experiment may be summarized as follows: A 1 per cent. solution of a soap, such as sodium olate, converts pneumococci into a viscid mass. Weaker solutions (.1 per cent.) do not kill the cocci, but they are more readily autolysed after the treatment. After exposure to still weaker solutions (1-20,000) the pneumococci show no changes in form or staining power, and are able to grow in cultures. But they are more readily autolysed, show increased susceptibility to the action of immune serum, and their virulence is somewhat lessened, although they are still able to produce septicemia in white rats.

If a series of rats are now inoculated the following general results (tabulated from Flexner's description) are obtained:

- Rats inoculated with untreated pneumococci, death in 18 hours.
- Rats inoculated with untreated pneumococci + immune serum, death.
- Rats inoculated with soaped pneumococci, death in 30 hours.
- Rats inoculated with soaped pneumococci + normal serum, death.
- Rats inoculated with soaped pneumococci + immune serum, recovery (animals not ill).

The soap and serum together were thus able to accomplish what neither could do alone.

The application of oleate and immune serum as a treatment of established pneumococcal infections meets with a serious difficulty, however, in the fact that the lytic action of soaps of fatty acids is prevented by the protein substances in the serum, and it is necessary to add a third substance such as boric acid to protect the soap from the protein. Flexner has applied this combination of soap, boric acid, and serum to the treatment of experimental pneumococcal meningitis in monkeys and has succeeded in thus curing the disease, from which untreated animals regularly die. An immune serum corresponding to the special strain of pneumococcus used is necessary to the success of the method. Pneumococcal meningitis in man has been treated by this method, but further study of the details of application is necessary before it can be brought into general use.
Morgenroth has devised a successful chemotherapy of pneumococcal infections in mice by means of ethylhydrocuprein. The combination of immune serum with the ethylhydrocuprein is much more effective than either alone. The percentages of recoveries of mice from intraperitoneal infection with the pneumococcus show the results of the combination of the two methods of attack. (Boehncke, 2.)

- Untreated: recovery in 0 per cent.
- Treated by chemical alone: recovery in 20 per cent.
- Treated by immune serum: recovery in 33 per cent.
- Treated by immune serum + chemical: recovery in 90 per cent.

A new field of usefulness is thus opened for specific immune sera, of which only a limited number have hitherto proved of unquestioned value in the treatment of the acute infections where their help is most needed. As Flexner suggests, an immune serum forms a very favorable basis on which to build up a specific chemical therapeutic agent, because the serum already has a structure suited to its union with the micro-organism, and is also relatively innocuous for the cells and tissues of the host.

Serum utilized as the carrier of an active chemical not only may make the chemical more effective, but may serve the further purpose of protecting special cells and structures of the body from the injurious action of the chemical. The employment of salvarsanized serum for the intraspinal treatment of tabes and paresis is a case in point.

The search may be long, however, before the combination of immune serum and an active bactericidal radical is obtained, which shall satisfy all the chemical conditions necessary that the remedy may sway unfailingly the balance of immunity against the invader. The problem involves chemical reactions of fine and intricate nature, and the solution for one disease may not be applicable to another disease having a closely related symptomatology. The mode of attack must be individualized for each disease, and may even have to be varied for stages of the same disease.

**THE INFLUENCE OF ONE INFECTION ON ANOTHER**

The chemical reactions involved in the struggle between the invading organism and the host are of an extremely intricate character, and the unstable balance between the two groups of forces may be swayed to one side or the other by many factors, some of which may be non-specific so far as we can tell from our present methods of determining specificity. The introduction into the subject of an infection of chemicals or cells which stimulate the production of leukocytes may suffice to influence the balance of the reaction toward recovery. The practical difficulty in the application of such vigorous and non-specific methods is met in the fact that the new element may swing the balance against the body as often as for it.
The experiments of Doerr, to be referred to later, show that the inoculation of bacteria or their toxins frequently renders animals much more susceptible to the invasion of other bacterial species subsequently introduced. The severe clinical course of multiple infections, by two or more organisms in the same individual, usually ascribed to the summation of the toxic effects of the organisms on the host, may be due to a coöperation of their combined ferments, or, speaking biologically, to a symbiosis, which enables them together to exert an aggressive action not possible for either alone. The secondary infections of tuberculous processes are instances of the unfavorable action of one infection superimposed on another.

Other combinations of diseases met with clinically offer examples in which the balance is deflected in favor of the host. Patients suffering from pernicious anemia may show a rapid improvement following an acute infection such as pneumonia or erysipelas. Certain malignant tumors may show a temporary arrest of growth, or may even decrease in size during and immediately after an intercurrent infection such as erysipelas. While the etiology of malignant tumors is a matter of controversy, it is generally admitted that they present in their immunological relations to the host many similarities to infectious processes, and it is easy to see that the balance between the aggressive forces of the tumor and the resisting forces of the host may be profoundly influenced by the introduction into the combined systems of forces of a third group derived from the acute infection. The chronic granulomatous process known as Hodgkin's disease presents a similar recession of symptoms under the influence of an intercurrent erysipelas.

NON-SPECIFIC INTOXICATION AS A CAUSE OF THE SYMPTOMS OF INFECTIOUS DISEASE

A number of problems arise in regard to the means by which the body rids itself of the infecting organism, and the part which this process of elimination plays in the production of the symptoms of disease. In the physiological process of gastro-intestinal digestion foodstuffs undergo successive stages of hydrolysis under the action of ferments, until they are resolved into substances sufficiently simple for absorption and assimilation. A similar process of splitting into simpler substances is assumed to take place when foreign protein substances are introduced into the body by parenteral routes, and the toxicity of some of these products produces a complex of symptoms known as anaphylaxis (q. v.).

Abderhalden (1) has extended his investigations of the relations of body cells and their specific ferments to the relations of the invading organism and the host. In order that the invading organism may gain a
foothold and multiply in the host it must possess ferments by which it can break down the substances of the host into products sufficiently simple that they may be utilized in building up the bacterial protein. If the organism does not possess such ferments it cannot obtain the necessary food supply, and hence is incapable of multiplication. The cells of the host may neutralize or otherwise prevent the action of the ferments of the micro-organism, and by this means the multiplication of the latter is prevented. Various drugs also may aid in the defense of the host by altering unfavorably the physical or chemical conditions of action of the ferment of the invader, or by changing the susceptibility of the fluids and tissues of the host to its action.

The host may suffer not only from the direct toxic action of the invader, but also from the possible toxic effects of the products of the proteolysis of his own tissues, brought about by the ferments of the invader. Finally the host suffers most severely from intoxication by the products of proteolysis of the foreign bacterial protein, induced by the ferments mobilized by the cells of the host in response to the stimulus of bacterial invasion. The identity and structure of the ferments of Abderhalden are as unknown as are those of the antibodies of Ehrlich, and we recognize their presence only by their effects on other substances. In studying the action of ferments, the physical and chemical changes in the substances on which they act, changes in rotation of polarized light and alteration of rate of diffusion through membranes, replace the phenomena of hemolysis, agglutination, and precipitation employed in the study of antibodies. (See Diagnostic Reactions.)

The phenomena of sensitization and allergy were first studied in animals following repeated inoculations of alien sera, but the principles of immunization developed from these facts have found a wide application in relation to the disturbances which follow the introduction of bacterial protein into the animal body.

The toxic action of bacteria was formerly ascribed to endotoxins liberated by the dissolution of the bacteria cells in the body. While endotoxins may be present and give rise to some of the toxic effects of bacterial infection, the view has been advanced that the products of digestion of bacterial protein itself are responsible for many of the toxic effects on the animal body. Vaughan, Friedberger, and others have shown that if a bacterial suspension is digested by chemical means, or by treatment with bacteriolytic sera, the toxicity of the suspension is enormously increased. The injection of suitable doses of these toxic products into normal animals produces symptoms of cutaneous irritation, respiratory embarrassment, hemorrhages, and death, identical with those produced by inoculations of the unaltered bacterial or other proteins into animals sensitized by a previous inoculation of the corresponding protein. This toxic substance has been called by Vaughan "protein poison," and by Friedberger "anaphyla-
toxin." The latter has also shown that if the proteolytic digestion is allowed to continue after the period of maximum toxicity is reached the products become less and less toxic.

Other writers, following the lines suggested by the work of Bordet, have found that by mixing serum with kaolin substances are produced equally as toxic as those derived from mixtures of serum and bacteria, and from these experiments have argued that the toxic substance is probably derived from proteolysis of the serum itself rather than from the bacteria.

Vaughan (13) has summarized the results of his experiments of the past ten years on the products of proteolysis of bacterial protein. Vaughan reports that he has been able to isolate a toxic substance from the cells of a number of bacterial species and also from vegetable proteins such as edestin and zein, which, in doses of .5 mg. given intravenously, is fatal to guinea-pigs, and in non-fatal doses when given to guinea-pigs produced a series of phenomena characterized by cutaneous irritation, urticaria, and later partial paralysis and also shallow rapid breathing, with a considerably marked depression of temperature. Small doses of the poison given subcutaneously will cause fever, as will also the unchanged proteins.

By regulating the size and interval of doses of the poison various types of intermittent and continued fevers can be produced. In those of the long-continued type progressive emaciation occurs.

In man the protein poison causes general cutaneous hyperemia and urticaria.

The relation between host and invading organism may be re-stated in terms of nutrition and proteolysis. In order that the organism may gain a foothold and multiply it must be able to split and utilize the proteins of the host, and the host must not at the outset be able to destroy the organism (proteolysis, bacteriolysis).

If either of these conditions is not fulfilled infection cannot occur.

After the infection has been present for a time the body of the host elaborates ferments (antibodies) which act specifically in limiting the growth and accomplishing the destruction of the invader. But after the invasion has been checked the host has still to dispose of the foreign bacterial protein, and it is the products of this parenteral digestion which are thought to give rise to the severe toxic symptoms of many infections.

Thus, as Vaughan points out, during the incubation period of typhoid fever rapid multiplication of the bacilli is taking place and they are building up typhoid protein out of the tissues of the host, but there is no splitting of typhoid protein, and no symptoms of intoxication are evident. After a period of ten days the cells of the host are sufficiently stimulated
to form specific ferments with which to break up the typhoid protein, and
the protein poison begins to show its effect in the production of fever,
headache, and prostration.

It may be added that at about this time the specific ferments (anti-
bodies) of the host limit the further growth of the invader, and soon after
the bacilli disappear from the blood. The course of typhoid fever may be
regarded as consisting of two overlapping periods, the first concerned
largely with the invasion, and later the limitation of growth, of the invading bacillus, on the one hand, and the sensitization of the host, on the other,
and the second with the disposal of the foreign protein remaining after the
invasion has been checked. An acceleration of the proteolytic process
results in the liberation of excessive doses of the protein poison, with
severe intoxication and perhaps death of the host. In this way forces
otherwise protective become injurious to the defender.

On this theory acute infectious diseases may be thought of as involving
an organ specificity on the part of the invader and its protein, which de-
termines the localization and many of the clinical features of the disease,
and a non-specific intoxication which is a result of the toxic action of
products of foreign protein liberated by the proteolytic ferments of the
host. In pneumonia the chief localization is in the lung, although there
is a coincident pneumococcemia; in the exanthemata the skin and some-
times other organs, such as the kidney, are most affected.

This theory of the non-specific cause of the symptoms of intoxication
in infectious diseases need not imply a non-specific defense on the part of
the host. That part of the defense directed toward the limitation of growth
and ultimate death of the invader may be accepted as clearly specific. Also
the ferments which break up the foreign protein may be specific for that
particular organism, even though the products of their proteolytic action
possess qualities in common with derivatives of other proteins.

Nor does the acceptance of a non-specific protein intoxication as the
cause of some symptoms exclude the possibility of the presence and action
of specific toxins, though these may play a less important rôle than was
formerly thought. Thus herpes is commonly seen in pneumonia and epi-
demic meningitis, rarely in typhoid. Accelerated respiration is the rule
in pneumonia, even with limited lung involvement, but may be entirely
absent in typhoid involving the lung.

ACTIVE IMMUNIZATION—VACCINE THERAPY

The success of active immunization by the inoculation of bacterial
products depends primarily on the ability of the body cells to produce
specific antibodies in response to the stimulus of the inoculation. Ass-
suming that in a given case such antibodies are formed, their therapeutic
efficiency will depend, not only on their nature and mode of action, but also on other conditions which may make their action possible. Agglutinins and precipitins are frequently formed in response to inoculations, but so far as we know at present they have very little, if any, direct part in overcoming bacterial invasion. The efficiency of bactericidal substances will depend, among other conditions, on the susceptibility of the special strain of bacteria to such action, and the accessibility of the bacteria to the antibody action. In order that opsonins may play an effective part in the cure of infection the bacterial invader must be susceptible to opsonic action, and leukocytes capable of active phagocytosis of the opsonized bacteria must be available. If one of these conditions is not fulfilled phagocytosis will not be increased and the anticipated beneficial results of the inoculation, as far as this form of immunity goes, are nil.

If, on the other hand, the cells of the body are unable to respond to the inoculation with the formation of opsonin or other antibodies the introduction of bacterial products not only may fail to produce immunity, but in certain cases may even increase susceptibility to infection, or influence unfavorably an infection already present.

The present widespread use of bacterial products dates from the demonstration by Wright and Douglas of the relations of opsonin content of the serum to phagocytosis in health and disease. In the evaluation of methods of vaccine therapy we may properly inquire to what extent opsonins and phagocytosis are efficient in eradicating the infecting organism from the body, and to what extent the present use of vaccines is supported by scientific data which gave birth to it.

**The Opsonic Curve**

The opsonic index expresses the relation between the amount of opsonin present in one specimen of serum, compared with that in one or more other normal sera. The relation is expressed by a figure obtained by dividing the average number of bacteria taken up by the leukocytes in the mixture of bacteria, leukocytes and serum to be tested, by the average number taken up by leukocytes in a similar mixture containing normal serum.

For example, the number of staphylococci taken up by 100 leukocytes in a mixture of leukocytes, staphylococci, and serum of a patient suffering from furunculosis may be 200. In a similar mixture of normal serum under the same conditions 100 leukocytes may contain, say, 400 staphylococci. The opsonic index of the patient is $200 : 400 = .5$.

Wright found that from repeated determinations of the opsonic index a curve may be plotted which has the general characteristics of other antibody curves, and that the curve may be modified by the inoculation of small amounts of killed cultures of the organism causing the infection. The course of the curve depends on the amount and periodicity of the
inoculations. Thus small doses tend to raise the curve very slightly for 4 or 5 days. A larger inoculation causes first a depression of the curve below normal for one or two days (negative phase), and later a rise to a point considerably higher than before (positive phase). If the amount of the inoculation is still larger the depression (negative phase) is more marked and of longer duration, and may or may not (depending on the excess of dosage) be followed by a positive phase. The importance of small doses of vaccine, such as would give the maximum opsonic response, and the necessity of giving successive doses at sufficient intervals of time to allow of the development of the maximum reaction from the previous inoculation, were emphasized repeatedly by Wright. He was able also to correlate the changes in the opsonic curve with the sequence of clinical changes, and to show that where the dosage and interval of inoculations were such as to give the optimum opsonic response a favorable course of the infectious process followed.

These principles of therapeutic use of bacterial inoculations were developed from careful studies of, for the most part, subacute and chronic localized infections, in which the focus of infection was more or less walled off from the rest of the body, and in which phagocytosis occupied a prominent place in the pathological changes in the focus of infection.

It was further demonstrated that in some types of localized infection fluctuations in the amount of opsonins occurred either apparently spontaneously or following mechanical manipulations, such as massage of an infected joint or exercise. These fluctuations were ascribed to auto-inoculation of the body by the passage of bacteria or their products from the focus of infection to the unaffected portions of the body.

**The Relation of Opsonins to Recovery from Disease**

It is obvious that a method which proposes to hasten the cure of an infection through the increase of opsonins presupposes that the deficiency of these substances is the only, or at least the chief, factor which is delaying recovery.

**Localized Infections.**—In certain localized infections, such as infections of the skin (furunculosis, acne), some forms of gonococcal arthritis, and others, the clinical results seem to confirm the conclusions drawn from the laboratory studies of opsonins; and it is generally agreed that in these forms of infection the increase in opsonin supplies an important element necessary to the completion of a successful defense against infection. But even in this class of cases it is not proved that other antibodies, such as bacteriolsins, may not have some part in the development of immunity, though their part in the process is not readily demonstrable.

It is to be noted in this connection that mechanical disturbances which interfere with physiologic readjustment may play a considerable part
in the continuance of a localized infection. "Without wishing to question the general accuracy of Wright's brilliant observations on localized infections, we might venture to inquire whether many such infections are due to lack of immunity at all. May not these miscarriages of processes tending toward the complete elimination of bacteria, as Wright calls them, be due to a slight disarrangement of the immune forces in their attack? If, for example, as he states, 'a wound may be pouring out day by day an ineffective pus,' would not a local vaccination tend to withdraw some of this 'ineffective' pus to itself and restore the balance? In those cases of 'brawny swelling' in which there are too few leukocytes and too much serum, may not the free incision give the pent-up bacteria a better chance to multiply in the open wound and thereby stimulate leukocytes to the spot to form pus and thus restore the balance in the opposite direction? These questions indicate that at least some localized infections may be due to disturbances of a delicate equilibrium rather than to a deficiency of immunity, as we understand the term. It is of interest to note here that in such cases vaccination is, after all, merely a safer and surer way of doing something which the setons of former centuries did in an unsafe and less certain way. If the seton wound became infected with the right organism, which might happen through the blood, or by accidental infection from the wound, the vaccination became a continuous process and an outlet for 'superfluous' leukocytes was made while the immunity was being raised." (T. Smith, 12.) Smith very aptly points out also that the seton vaccination was a vaccination not only with living bacteria, but by autogenous organisms, providing the infecting organism gained entrance to the site of the seton.

**Generalized Infections.**—If we now consider the conditions present in a general infection or sepsis it is at once evident that they are in several essentials the opposite of those in a localized process. The infection is generally distributed over the body, and all the cells of the body are receiving the stimulus to antibody formation. The opsonic content of the blood may be continuously high, or continuously low, or may fluctuate through a wide range. If high, theoretically nothing is to be gained by a further increase over what we know to be an efficient quantity in local infections. If low, then from our knowledge of the development of opsonins in local infections only minimal amounts of bacterial inoculations are permissible, and these we know are likely to have but an insignificant effect on the total amount of opsonin present. The inoculation of larger amounts will serve still further to depress the amount of opsonin present. It has been suggested that the subcutaneous tissue cells are more efficient producers of opsonins than are the tissues generally, but the advocates of this view overlook the fact that the products of bacterial infection reach subcutaneous tissues quite as extensively in the course of sepsis as when introduced hypodermically at one point. The argument that suspensions of the
killed, but otherwise untreated, organisms are more efficient than are live bacteria is not supported by experiments in the active immunization of animals in which it is a general principle that small doses of attenuated, but living, organisms produce a greater immunity than can be obtained by killed organisms.

Further, the deficiency of opsonin is not the only cause of the persistence of a general infection. The opsonin content of serum of a patient may be persistently high up to the time of death, and patients with a continuously low opsonic index may recover. Some species of bacteria are insusceptible to phagocytosis, and in other species, usually phagocytably, special modifications of the organisms, such as capsule formation, and probably also specific chemical changes occur which render them immune to phagocytic action. Functionally active leukocytes also are necessary to successful opsonification. Finally the element of time must be considered in comparing the conditions of local and general infection. All our information in regard to antibody formation indicates that a definite period of time (usually several days) must elapse between the inoculation and the maximum development of specific antibodies. In chronic local infections this period is of small moment with respect to the total period of illness, but in acute sepsis it becomes a factor of great importance, for a therapeutic method must be able to give quick results in order to meet the immediate need of the patient.

It is thus evident that the facts which lead us to believe that in certain chronic local infections the failure of eradication of the disease is due to lack of opsonin, and that this lack may be remedied by the artificial increase of opsonin by specific bacterial inoculation, are not scientifically demonstrated in the case of general infections, and the treatment of general infections by inoculations for the purpose of stimulating the production of opsonins is unsupported by the laboratory evidence at hand. If inoculations of preparations of bacterial substances are used in general infections this use must be based on other evidence independent of opsonification, and only after carefully conducted experiments.

Infections Not Strictly Localized.—Midway between the general infections associated with bacteriemia on the one hand and the strictly localized infections on the other, stand a large group of clinical conditions exhibiting more or less localization, but subject to occasional temporary bacteriemia, as evidenced by changes in the general clinical symptoms, the intermittent presence of bacteria in the blood, and the formation of new metastatic lesions. The opsonic content of the blood in these cases fluctuates through a wide range and relatively slight physical changes, such as movement of joints, exercise, the application of a bandage, massage (auto-inoculation), may result in a rise or fall in the opsonins. Here the balance between the aggressive forces of the invading organism and the protective forces of the host is a very delicate one, and a relatively slight
impulse may serve to throw it to one side or the other. This delicacy of adjustment, evident serologically as well as clinically, should call for the greatest care in the use of measures calculated to disturb the balance. In the present use of vaccines, however, there is no principle which has been so recklessly disregarded.

The cases in this middle class fall into two general groups with respect to the acuteness of the process. In the more acute group may be mentioned the phlegmons, lymphangitis, lymphadenitis, acute osteomyelitis, acute gonococcal arthritis, acute empyema and peritonitis; in the more chronic group may be placed chronic colon and tuberculous infections of the urogenital tract, tuberculosis of bones and joints, pulmonary tuberculosis, and some forms of chronic gonococcal arthritis. Other examples will occur to the reader. The more carefully the acute forms of this group are studied the more often are bacteriemias demonstrated, and, although a temporary invasion of the blood is undoubtedly frequent in cases presenting the features of a localized infection, for the time at least the relations of invader to host approach very closely those obtaining in a general infection.

Another feature of these cases is the frequency with which they call for active surgical treatment, the need for which should not be neglected in attempts to stimulate the bodily immunity by inoculations. The incision and drainage of an abscess, or the excision of a focus of infection may be all that is necessary to accomplish a rapid cure. It is often wiser to take off part of the load than to attempt to whip up the fagged horse.

Thus far we have considered some of the arguments which have been advanced in favor of the view that infectious processes fail to heal by reason of a deficiency of opsonins, and that an increase in opsonification is the only factor necessary to attain such healing.

In certain infections, particularly those strictly localized, but occasionally in the less strictly localized, an increase in opsonic power of the serum apparently furnishes the element necessary to recovery.

At this point we may note that if a series of localized infections are studied with special reference to the results of inoculations it is found that the most favorable results are obtained in those cases which present an intermittent type of infection, such as occurs in furunculosis. Here the disease may be regarded as a series of acute localized infections, and the treatment as a prophylactic inoculation against subsequent attacks (furuncles) rather than curative of the lesions which are already existing.

The Effects of Inoculations on Antibodies Other than Opsonins

In addition to the increase of opsonin we have still to consider some of the other possible effects of bacterial inoculations on the antibody con-
tent of the serum. It has already been pointed out that, while the chief
demonstrable effect of inoculations in some localized infections is an in-
crease in opsonin with resulting increase in phagocytosis, a coincident
increase in bactericidal properties of the serum cannot be excluded, and
indeed may be readily shown. In general, the methods available for the
demonstration of the bactericidal qualities of a serum are less delicate, and
so such substances may vary in amount without their change being de-
tectable by the laboratory methods at our disposal. The fallacy of at-
ttempting to account for all the changes in immunity toward an infection
by studies of opsonins is as obvious as that of trying to make the special
combination of forces by which diphtheria is cured by antitoxin apply to
the cure of all infections.

The unfavorable action of bacterial inoculations must also be con-
sidered. The danger of producing a prolonged negative phase by too large
or ill-timed inoculations and the coincident bad clinical effect has been
emphasized by Wright and others. Less attention has been paid, however,
to the possible harmful effect of the inoculations on bactericidal and other
defensive antibodies. In considering the effects of bacterial inoculations
on immunity the experiments of Bail may be recalled, in which he found
that the bacteria-free peritoneal exudate of a guinea-pig killed by repeated
intraperitoneal inoculations of typhoid bacilli when introduced with
typhoid bacilli into a second animal, enabled an otherwise non-fatal dose to
result in a fatal infection of the animal. While the conclusions of Bail as
to the nature of the substance in the exudate called by him "aggressin"
have been largely disproved, the experimental fact remains that substances
probably allied to, or identical with, bacterial endotoxins may help to break
down a preexisting immunity. Substances similar in action have been
obtained by autolysis of bacteria in distilled water (Wassermann and
Citron) and various filtrates of bacterial cultures including toxins derived
from the diphtheria bacillus and cholera vibrio enable otherwise non-fatal
doses of bacteria to cause fatal infection (Doerr).

These few facts and others which might be cited are sufficient to show
that from the standpoint of experimental medicine the treatment of in-
fec tious processes by bacterial inoculations has some, though a very
limited, support, and such methods must be used with extreme caution.
Much might also be said of the effects of bacterial products on the physi-
ological processes of the body, quite apart from the question of the infecting
organism. The relation of chronic infection to nephritis, amyloid changes,
myocardial and other degenerations has long been recognized.

The broad principle that the animal body has the power of reacting to
infectious processes by the formation of specific antibodies forms the basis
of our entire conception of immunity, but the mode of the reaction, the
type of antibodies formed, and the combinations with other substances by
which these antibodies become effective differ in various infections, so
that each infection must be studied separately. Our available information based on experimental evidence is greater in some types of infection than in others, but in none is our knowledge at all complete, and methods of artificially increasing immunity in one infection may be entirely inapplicable to another.

It is generally accepted that any proposed treatment should be based on adequately controlled animal experimentation. Unfortunately, a number of infectious diseases of man present conditions of infection and susceptibility which cannot be duplicated in animals by any means at present at our disposal, and as a result in these diseases specific therapeutic measures have developed slowly, or are still unattained. The impossibility of animal control cannot be accepted as license for empiricism, however, and in the evaluation of any method the burden of proof thrown on serological and other laboratory methods on the one hand, and on clinical observation on the other, is greater and not less by reason of the lack of available animal experimentation.

The Clinical Evidence For and Against Active Immunization

In judging of the clinical results of any kind of treatment careful regard must be had for the normal course of untreated cases; the greater the number of clinical possibilities in the unmodified disease the more guarded must be conclusions drawn from the course of treated cases, and the larger the series of observations necessary for the formulation of conclusions. Disregard of this principle is not peculiar to the use of specific biologic products, but nowhere in medicine has neglect of it led to greater absurdities and often to harm.

A retrogression of symptoms is often striking evidence of the success of a therapeutic measure. If such improvement occurs regularly after a given treatment when used in cases in which improvement does not usually occur so quickly we have some reason to believe that the measure has therapeutic value. In any individual case the question as to whether such improvement has occurred is necessarily decided by the judgment of the physician, whose conclusions are based in turn on his experience with other similar cases and on the accuracy of his observation. There are, however, so many undeterminable and unrecognized factors which influence the outcome of any individual case that case reports covering a small number of observations offer very little assistance in determining the value of a method, particularly when we remember that there is always a tendency to report favorable cases, and to allow the unfavorable to pass unnoted. Only when large groups of cases accompanied with adequate control cases are available can clinical evidence approach in reliability that of animal experimentation.

Vaccines in Localized Infections.—The efficacy of inoculation of bac-
terial products in prophylactic immunization against certain diseases may be accepted as established clinically, as well as supported by serological and animal experiment. In certain strictly localized infections, too, there is some clinical evidence that the infectious process may be favorably influenced by bacterial inoculations. Even here, however, it is by no means established in all cases that the favorable outcome is due solely to an increase in any one class of specific antibodies. It is altogether likely that the defensive action of the body is multiple rather than single in most forms of infection. It has already been pointed out that some of the localized infections of which furunculosis is a type may be thought of as a series of acute infections, and that the inoculation of vaccine accomplishes a prophylactic immunization against succeeding attacks (new furuncles) rather than the cure of preceding lesions.

The healing of a localized infection following the use of vaccines cannot of itself, however, be taken as proof of the efficiency of the inoculation. Acute localized infections frequently heal spontaneously and favorable results in this class of cases must be discounted accordingly.

**Vaccines in General Infections.**—Under general infections may be included those diseases such as pneumonia, typhoid fever, staphylococcus and streptococcus sepsis, in which there is a profound disturbance of the physiology of the entire body as evidenced by fever, and other signs of sepsis, and in which a more or less persistent bacterial invasion of the blood is demonstrable. The treatment of this class of cases by vaccines, as the term is generally accepted, has not been followed by clinical results which justify the method as a routine procedure. It is true that the nature of the clinical evidence is not the same in all diseases of this class, and that in certain cases vaccines apparently have modified the course of the disease in a favorable way. Thus the administration of typhoid vaccines has in some cases seemed to modify favorably the course and complications of the disease as compared with untreated control cases. (See Typhoid Fever.) In puerperal sepsis adequately controlled case reports are few, and the consensus of opinion has been that the favorable outcome of the treated cases which recovered could be attributed as well to the normal variability of the disease as to the specific method of treatment. The periods of relative comfort and low temperature, so often attributed to the favorable action of vaccine, in the hours immediately following the "reaction," are in no sense indicative of the success of the inoculation, if for no other reason than that they are frequently observed after a chill and temporary exacerbation of the untreated disease, and form one of the well-recognized clinical symptoms of sepsis.

The bad effects of introducing toxic substances into a body already overtaxed with substances of the same nature may be as evident to the careful clinician as to the serologist. But too often they are attributed to unforeseen turns of the disease, rather than to their true cause.
In ulcerative endocarditis inoculations of vaccines almost always have proved ineffectual in staying the fatal outcome of the disease. In rare instances ulcerative endocarditis has been thought to heal spontaneously, so that it cannot be said to be an absolutely fatal disease. After observing a fairly large series of cases under his own care and that of others, the writer has yet to see an instance in which the use of vaccines has had any lasting beneficial effect, or has prevented the final death of the patient.

**Vaccines in Infections Not Strictly Localized.**—As indicated, between the class of strictly localized infections and those clearly general in character, there is a very large group of borderline cases which present features characteristic of both the others.

The clinical data on the efficiency of vaccines in this borderline class are unsatisfactory, as might be expected in a group containing infections which in one case may be localized almost completely, and in another may border on a continuous bacteriemia, with little or no clinical evidence of immunity. Medical journals are filled with reports of such imperfectly studied and uncontrolled cases, from which wildly enthusiastic conclusions are drawn. The more one studies this class of cases the more cautious does he become in his use of bacterial inoculations, and the more inclined to the view that unless they can be subjected to careful individual clinical and immunological study these cases should not be treated by vaccines.

In summarizing the indications for and against the use of vaccines I cannot do better than quote the words of Theobald Smith:

“All parasites tend to increase the resistance of the host in which they live and multiply. Out of this universal fact a number of practical problems arise. In any given disease is it worth while to try to raise this immunity, and how much energy will it cost the patient? If worth while, what is the best and most sparing way of raising such immunity artificially? In any localized infection we must ask: Is this a beginning process without attendant immunity, or is it a residual process associated with general immunity? If the latter, then vaccines may be considered safe. In processes associated with fever and bacteriemia science says ‘Hands off’ until we know whether we have a progressive disease with gradual undermining of the resistance, or a more localized affection in which the excursions into the blood are secondary. In any case, the use of vaccines in these cases must be regarded as experimental and should not be undertaken save in conjunction with one trained in immunologic problems.

“Judged from this point of view, as well as from the work of the laboratory, we should say that vaccines applied during disease will be rarely, if ever, life-saving, but they may hurry a stationary or languid process which tends toward recovery, by bringing into play the unused reserves of various tissues.”
Reactions Following the Injection of Bacteria or Their Products

The inoculation of specific bacteria or their products into an individual suffering from an infectious disease is followed by clinical changes in the subject which constitute what is known as "reaction." These changes may be (1) local, at the site of the inoculation (erythema, edema, necrosis); (2) focal, at the site of the localization of the infection (evidences of new activity of the infection, increase in pain and swelling in joints, increased secretion with râles in pulmonary tuberculosis); (3) general, increased fever and leukocytosis, malaise, headache and occasional functional disturbances in organs not primarily involved in the infectious process. Clinical reactions have been extensively employed in diagnosis (cf. Diagnostic Reactions), and have proved of some service in the regulation of the dose and interval of inoculation in the treatment of disease by active immunization. The relation of these reactions to other allergic phenomena is discussed in a succeeding chapter (cf. Anaphylaxis).

The Neglect of Surgical Procedures

The inoculation of bacterial products for the cure of local infections, like other new procedures in medicine, has been employed under conditions not contemplated by those who devised it, to the neglect of older and approved methods of treatment. The attempt to promote the healing by inoculations of vaccines, of an acute abscess, in which incision and evacuation of pus is all that is necessary, shows a total misapprehension of the principles of therapeutic inoculation, and, besides, is wasteful of the time and energy of the patient.

Chronic metastatic infectious lesions arising from some primary local suppurative focus, such as may be found in tonsils, sinuses, alveolar abscess or gall-bladder, call first for surgical treatment of the primary lesions, and when the focus of infection is removed the natural resistance of the body is usually sufficient to overcome the remaining infection.

Sensitized Vaccines

Bacterial suspensions which have been exposed to the action of a corresponding immune serum undergo certain modifications which result in changes in their immunizing qualities when inoculated into animals. These modified bacteria are termed "sensitized vaccines." The method is based on the principle enunciated by Ehrlich and Morgenroth that cells, whether bacterial or animal, are able to fix or attach to themselves their corresponding antibodies, and that this union of cell and antibody persists when the cells are washed free from serum. Although combinations of
bacteria and immune serum had been used for immunization by other workers, Besredka, in 1902, was the first to use the method extensively. Much of the experimental work on sensitized vaccines has been done in relation to immunization against typhoid fever by means of sensitized living typhoid bacilli, but the method has been extended to apply to the treatment of typhoid and other bacterial infections. Besredka, working with monkeys, has found that immunization with living sensitized typhoid bacilli produces an immunity which is superior to that produced by other methods, and believes that the procedure is devoid of the danger of producing typhoid carriers.

Garbat and Meyer (5) immunized rabbits with repeated injections of unsensitized typhoid bacilli, and with bacilli sensitized with immune rabbit serum. They found that the animals which received unsensitized bacilli exhibited severe toxic symptoms, with diarrhea, and frequently died after the third injection with inflammatory lesions in the intestine which were interpreted as phenomena of anaphylaxis. The animals treated with sensitized bacilli presented symptoms less severe, the initial rise in temperature lasted a shorter time, though it reached a point somewhat higher than in the animals receiving unsensitized bacilli. The serum from the animals treated with unsensitized bacilli possessed a much higher agglutination and complement-fixing antibody titre than did that from the animals receiving sensitized bacilli. Nevertheless the protective properties of the serum from sensitized bacilli when introduced into guinea-pigs and mice following the injection of typhoid cultures were much greater than in the case of the serum obtained by inoculations of unsensitized bacilli. When the two sera were mixed, and the protective power of equal volumes of the mixture and of each serum separately compared, it was found that the mixture was able to protect in smaller volume than the same amount of either serum separately.

Broughton-Alcock (3), working in the Pasteur Institute in Paris, has used sensitized living vaccines in typhoid immunization in man. The method of preparation of the vaccine is as follows: A 24-hour agar culture of typhoid bacilli is washed off with 1 c. c. of .8 per cent. salt solution and .5 c. c. of antityphoid horse serum is added to the suspension. The mixture is allowed to stand 24 hours at 20° C. Salt solution is added and the suspension centrifuged five or six minutes. The supernatant fluid is discarded, and the precipitated bacilli again washed with salt solution and centrifuged. After again making up the suspension to 10 c. c. with salt solution, it is distributed in amounts of 1 c. c. to 10 tubes of 9 c. c. each of salt solution. One-tenth of 1 c. c. of one of these test tube suspensions contains about 500,000,000 bacilli, the minimum first adult dose. The sensitized bacilli stain well, have a regular cell outline, but appear broader and apparently swollen. The sensitized bacilli remain alive in these emulsions for some time, and living bacilli have been found as long as
four and one-half months after preparation. Multiplication of bacilli has been observed in some preparations.

Patients treated with sensitized typhoid vaccine showed very little increase in agglutinins as compared with patients immunized with vaccine prepared in the ordinary manner. Ehrlich has accounted for the lack of agglutinins on the ground that the bacterial receptor which fixes on the cell and calls forth agglutinin is already occupied by substances from the immune horse serum, and only in rare instances is this combination dissolved. Fixation of complement also was not observed in the serum of patients treated with sensitized vaccines, while the serum of four patients treated with non-sensitized vaccine fixed complement on the eighth day after inoculation. Broughton-Alcock suggests that the best test of immunity is the history of immunity in the patients rather than the detection of immune bodies in the serum, and supports his argument by the well-known fact that, after an attack of typhoid, immune bodies soon disappear from the serum so far as we know from our present methods of detecting them, but the immunity persists for years, or life.

In his first series of inoculations, Broughton-Alcock observed no general disturbances and only insignificant local reactions. In one case an abscess developed at the site of inoculation from which were isolated the typhoid bacillus and a staphylococcus. In all, about 750 persons were immunized with satisfactory results.

The employment of a living sensitized virus for immunization undoubtedly approaches more nearly to the theoretically ideal conditions for the development of immunity, as seen in vaccination against small-pox. However, the question properly may be raised as to whether in typhoid immunization such a near approach is necessary to obtain practical results. As pointed out by Russell, many of the European failures to obtain satisfactory immunization with ordinary killed typhoid vaccines were due to improper methods of preparation, such as overheating of the suspensions.

The strongest argument for the efficiency of ordinary vaccines in typhoid immunization is the brilliant success of the method in our own army. So far as the production of an immediate, efficient immunity is concerned, no modification of method can give much better statistical results. Whether the immunity conferred by sensitized vaccines will be more lasting can be determined only by observations over long periods of time. It is possible that the use of sensitized killed typhoid vaccines may decrease the number of severe reactions, and thus prove an advantage of sensitized over unsensitized vaccines. Further, it may be noted that the substitution for heat, of phenol or other similar substances may offer advantages in immunizing power similar to those of sensitization by serum. Gay and others have used sensitized typhoid vaccine extensively for both immunization and treatment (see Typhoid Fever).
TREATMENT BY INTRAVENOUS INJECTIONS OF FOREIGN PROTEIN

The treatment of disease by intravenous injections of homologous and of foreign proteins including those of bacterial origin has recently occupied the attention of both the laboratory and the clinic, and the physician is confronted by the question as to how far he shall utilize these methods in the treatment of patients in his charge. The answer to this query is difficult. Two questions seem to be involved: one, as to what extent the phenomena of the reaction are part of the mechanism of the recovery from disease; the other as to whether, in the present state of our knowledge, practical results justify the use of the method in the treatment of patients.

In brief, the phenomena which follow the intravenous injection of bacterial protein or of albumose are a rise in temperature with rigor, and more or less profound general symptoms, such as rapid pulse, cyanosis and marked increase in leukocytosis, followed by a drop in temperature. Changes in serologic reactions with respect to opsonins or agglutinins have been noted. When such reaction is provoked in persons suffering from typhoid or other febrile disease the fall in temperature may be permanent, as in the crisis of pneumonia, and convalescence seems in certain cases to be hastened.

Typhoid fever, puerperal sepsis, pneumonia, erysipelas, arthritis of various forms, are some of the diseases which have been treated in considerable numbers by this method.

Before considering some of the questions of therapeuseis which are suggested in these interesting reactions, it is desirable to point out in what respects they promise to modify the hitherto accepted views of immunity.

Specificity in biologic reactions has been the guiding principle of those who have attempted to devise methods of therapy in infectious diseases. The theory of antigen and antibody, developed by the genius of Ehrlich, has dominated therapeutic research in infectious disease up to the present time. His conceptions of strict specificity in the relation of the reaction products of the animal body to foreign protein, whether of bacterial or other origin, have been repeatedly questioned, but while his theories have required modifications to meet the requirements of the physicochemical principles, the fact remains that it has been under their guidance that our most valuable specific remedies, such as antisera for diphtheria, tetanus, and meningitis, salvarsan, prophylactic immunization against typhoid, and diagnostic aids based on agglutinins, precipitins, and complement fixing substances, have been developed.

In considering the possible non-specific elements in the phenomena of disease, it is not proposed therefore to supplant the older specific conception, but to discover in what ways these non-specific reactions help or hinder the specific factors, or act independently of them.
It is also important to bear in mind that the specific biologic reactions which present so high a grade of specificity that we have come to regard them as something apart from ordinary chemical reactions, are merely instances of physicochemical processes, whose fine degree of adjustment can be attained only under conditions met with in the animal body. The phenomena of agglutination, phagocytosis, and precipitation, can all be produced artificially—though less perfectly than by using materials from the animal body—by properly adjusting and modifying the concentration of electrolytes, or the surface tension of nonmiscible fluids.

And so no matter what the ultimate conclusion as to the nature of the nonspecific reactions may be, it is altogether probable that they follow the same chemicophysical laws as reactions which we have come to regard as highly specific.

The first question we may ask is: In the course of and recovery from infectious disease, to what degree are specific and nonspecific factors concerned?

In typhoid fever the specific factors so far as we know them seem to be opsonins, lysins, and from recent studies the agglutininins have also been included.

Of the nonspecific factors in the production of the symptomatology of typhoid we note that Vaughan years ago suggested that the fever of typhoid was caused by products of splitting of typhoid protein, and he was able to produce various types of fever in animals by the injection of protein split products obtained by hydrolyzing bacterial protein of colon or other bacteria, or egg white. Phagocytosis, both endothelial and leukocytic, seems to be important. Polynuclear phagocytosis is facilitated by specific opsonins, and Gay has maintained that the hyperleukocytosis following intravenous injections of typhoid bacilli is also specific in that it is more pronounced in animals previously immunized than in non-immunized animals. Other investigators have failed to confirm Gay's work in this respect, and find that the hyperleukocytosis is nonspecific and can be elicited in untreated as well as in treated animals. The phenomena of the reaction are not peculiar to patients suffering from febrile disease, for the same reaction has been observed in normal persons injected intravenously with typhoid vaccine.

Several theories have been advanced to explain the change in the clinical picture and disappearance of previous symptoms of disease following intravenous injections.

Jobling and Petersen have shown that the serum protease of the blood is increased by intravenous injection of protein, and suggest that the ferment normally held in check by antiferment is freed by the removal of the latter, and at once acts on the toxic fever—producing substances in the body, breaking them down to nontoxic elements.

The hyperleukocytosis which accompanies the reaction has also been
held to accelerate the phagocytic removal of invading bacteria. This seems to be largely a nonspecific factor.

There is also some evidence to indicate that the reaction produces indirectly a specific effect by mobilizing or calling into action specific immune substances which have already been formed, but have not developed their maximum effect in the body of the patient.

Perhaps the most important question to all is—To what extent may those nonspecific methods be at present employed in therapeusis with profit and at the same time safety to the patient?

It must be evident that our knowledge of what takes place in the body of the patient is at present largely conjectural. Clinically, on the one hand we have reports of rapid defervescence and apparent improvement in some of the cases of typhoid, and of subsidence of arthritic phenomena in patients suffering from infectious arthritis, following the intravenous injection of foreign protein. On the other hand, many patients derive no demonstrable benefit, and in still others the improvement noted is ascribable to the natural course of the disease as well as to the treatment. Nor can we overlook the severe reactions, the instances of cardiac dilatation, and also the occasional though rare deaths which have at least followed, if they have not been due to the injections.

The study of the nonspecific phenomena of disease is of great importance, perhaps greater than we now realize, and promises to open for us new avenues of attack by which we may hope to arrive at a better understanding of the way in which recovery from infectious disease occurs.

It is possible too that methods may be found to obtain the favorable nonspecific effects without exposing the patient to the rigorous experiences of present methods.

From the point of view of safe therapeusis, however, it would seem well for the present to maintain great conservatism in employing these methods in the treatment of the disease even under circumstances which permit of careful study and control. Their general and indiscriminate use is to be condemned.

The balance of immunity is a very delicate one, which may easily be deflected for or against the patient, and we shall not wish to alter this balance unless we can be sure that the change will be uniformly in favor of the patient.

REFERENCES

THE PRINCIPLES OF SPECIFIC THERAPY

10. ——. Ibid., 1913, i, 708.
CHAPTER II
THE FUNCTIONAL ANALYSIS OF ANAPHYLAXIS

JOHN AUER

Introduction.—One of the most important scientific achievements of the last decade has been the development of the conception called anaphylaxis, or protein hypersensitiveness, and on its basis insight has been gained into many puzzling processes, especially in the so-called idiosyncrasies. Any living structure of the organism apparently may be the seat of anaphylactic changes, and these changes manifest themselves according to the nature of the structure affected, which may be, for example, the skin, the respiratory tract, the circulatory apparatus, or the gastro-intestinal canal and its various glands. This variety of effects and affected structures makes the study of anaphylaxis of great importance to the physiologist, pharmacologist, and to the modern clinician. But this variety of effect emphasizes an important point in our conception of anaphylaxis, and that is that anaphylaxis is not a clinical entity as far as symptoms go, like outspoken cases of lobar pneumonia, acute articular rheumatism, or exophthalmic goiter, to be diagnosed by a series of well-defined symptoms, but that anaphylaxis is an entity only when viewed as to its causation.

The various theories and theoretical applications of anaphylaxis will be touched but lightly. No attempt will be made to give an exhaustive survey of the subject, but attention will be paid chiefly to the functional changes which anaphylaxis calls forth in the animal organism, for such alterations are the phenomena which the clinician meets in his daily work. As a large part of our information about anaphylaxis has been gained through animal experimentation, much of what follows will deal with the lower animals, because only in them could the investigator carefully and laboriously study the causation of the disturbances produced. While caution must undoubtedly be exercised in transferring the results gained by animal experimentation to the explanation of the disturbances observed in man, it is not premature to state that many, if not all, the typical anaphylactic phenomena observed in the guinea-pig, rabbit, and dog apparently find their counterpart in man.

The restriction in the scope of this article is all the more permissible because a number of excellent general reviews and presentations of spe-
pecific viewpoints are available to the student of anaphylaxis (see References).

Historical.—Like all advances in science, the conception of anaphylaxis was foreshadowed many years ago. According to Morgenroth, the famous French physiologist Magendie noted that rabbits tolerated an intravenous injection of egg-albumen, but succumbed when the injection was repeated after an interval of days. A similar observation was made by Flexner in 1894, who found that rabbits survived the intravenous injection of dog serum, but died when the same serum in the same amount was again injected after an interval of days or weeks. The observations of Behring in 1893 are also of interest in this connection, although their identity with anaphylaxis is not yet fully established. Behring observed that horses, sheep, and goats, which had been immunized with diphtheria toxin or tetanus toxin, became in time so sensitive to the injection of the substance that they succumbed to a small fraction of the dose which normally caused only a transitory reaction; at the same time it was demonstrated that their serum showed a higher content of antitoxin. Similar and still more striking results were observed by other investigators in guinea-pigs. In spite of the interest of these observations the existence of a new principle was not suspected and the subject became important only after the researches of Richet, Arthus, v. Pirquet and Schick, Theobald Smith, Otto, and Rosenau and Anderson.

The researches of Richet (102) and his collaborators, which began as early as 1902, brought out a number of valuable facts. He used water extracts of the tentacles of sea anemones, actinia and mussels, and also a vegetable toxalbumin, crepitin. Although all these extracts were poisonous, and in proper dosage caused death, Richet found that sublethal doses, which produced but mild symptoms in normal dogs, would elicit violent effects and death when injected intravenously into dogs which had received a similar injection two or three weeks previously. A cumulative action of the poison was ruled out by the observation that a reinjection after three to five days produced only a moderate effect. Moreover, Richet (99) found that normal dogs could be rendered highly sensitive to these extracts if they were first injected with the blood of animals which had been injected with these substances. There was, therefore, something in the blood of treated animals which transmitted this state of increased susceptibility to the action of the poisonous extracts. These experiments showed not only that a certain time was necessary between the injections before the animal would exhibit this enhanced reaction, but also that a state of increased and transmissible susceptibility to the action of the extracts had developed. In order to emphasize this, and to bring out clearly the point that the injected animal had not developed an increased resistance to the injected substances, but on the contrary had become more sensitive to it, Richet coined the word anaphylaxis.
The symptoms observed were briefly as follows: The reinjected dog shows within a few seconds dyspnea, vomiting, general weakness, and diarrhea. Associated with this is a strong drop in blood pressure. (Richet, 98.) Death occurs in a large percentage of the dogs within one hour after the reinjection. Similar effects were also observed in rabbits which had been previously injected with actinia extract. He also noted that the animals reacted most strongly when the same extract was injected which was employed the first time.

The most important facts contributed by Richet were that the injection of dogs and rabbits with small, almost harmless doses of poisonous albuminous extracts produces, after a definite and necessary interval, a state where the injection of the same dose causes an immediate violent intoxication which often leads to death. This state of hypersensitiveness, or anaphylaxis, could be transmitted to normal animals by injecting them with the blood of dogs which had previously been treated with the extracts.

Richet's experiments were, however, complicated by the material he employed. Because the extracts utilized contained both a toxin and a protein, his animals showed at the same time an immunity to the toxin due to antitoxin formation and a hypersensitiveness due to the proteid portion of the extract injected. On reinjection, therefore, Richet (100) sometimes observed that the prepared dogs showed an initial intense effect, but nevertheless survived, although the dose was so large that normal dogs invariably succumbed.

This complication of the experimental result which Richet's work shows was avoided by Arthus, to whom we owe the first physiological investigations in anaphylaxis produced by the injection of a non-toxic serum. He demonstrated that horse serum,¹ fresh or preserved, heated to 57° C., or unheated, could be injected subcutaneously, intraperitoneally, or intravenously into rabbits without causing any immediate or remote accidents. If these injections are repeated every six days, however, the rabbit sooner or later reacts with a pronounced and striking skin reaction if the injections are given subcutaneously or with more or less profound general symptoms when the last injection is given intravenously. Arthus (2) also noted phenomena of anaphylaxis in the guinea-pig and the rat after they had been injected repeatedly with horse serum. He was also able to produce similar effects when milk or egg-albumen was used instead of horse serum.

Arthus was the first to show that an originally harmless protein may produce grave toxic symptoms, and even death, when injected repeatedly at certain intervals into an animal, but he did not regard these reactions as specific. (Arthus, 2, 3.)

Shortly after the first publication of Arthus, v. Pirquet and Schick (96)

¹The horse sera used by Arthus were the antitoxins for diphtheria, tetanus and snake venom.
reported the results which they had obtained when rabbits were reinjected with horse serum. Their investigation was undertaken in order to gain an insight into the causation of the morbid changes which sometimes occur in man after the injection of diphtheria antitoxin, for example, fever, urticaria, edema, painful swelling of the joints, etc. In 1905 a monograph appeared by the same authors dealing with these complications which the authors call serum disease (v. Pirquet and Schick, 97). In 1903 Theobald Smith made his first observations of the phenomenon which was later to bear his name. During the routine examination of diphtheria antitoxin to detect any possible bacterial contamination, and to determine its antitoxic titre, Smith noted that guinea-pigs which had survived the injection of a diphtheria toxin-antitoxin mixture frequently died within a few hours when the antitoxin was again injected. For normal guinea-pigs, that is, pigs which had never before been treated with antitoxin or with toxin-antitoxin mixtures, the injection of antitoxin was nearly always harmless.

Theobald Smith's observations were fully corroborated and amplified in 1906 by Otto, and independently by Rosenau and Anderson. Otto investigated the "phenomenon of Theobald Smith" at the request of Ehrlich, whose interest in the subject had been aroused by a conversation with Theobald Smith in the latter's laboratory. Rosenau and Anderson (107), of the United States Public Health Service, investigated the question in order to gain information about the cause of sudden deaths which now and then occur after the administration of diphtheria antitoxin in the human being.

These important researches, which will be considered more in detail later, roused general interest and ushered in the general experimental study of anaphylaxis, for investigators now had a definite procedure and a highly suitable animal, the guinea-pig, at their disposal.

**EXPERIMENTAL ANAPHYLAXIS**

The term anaphylaxis is employed in this article in the following sense: It is used as a group name for those alterations of function and anatomical changes which occur when an animal is reinjected after an appropriate interval with the same protein solution; these alterations must not be obtained when the same dose of the protein is injected into a normal animal. Attention must be called at once to the important fact that the reactions observed in the anaphylactic animal are not in themselves diagnostic, for similar and even identical reactions can be obtained with a large number of widely different substances; but these reactions, when they occur for the first time as the result of a definite procedure, viz., reinjection of the same protein after a definite interval, are then absolutely characteristic of anaphylaxis.

The term anaphylaxis is employed, by some, to designate the sensitized
state, that is, the state which occurs after the animal has been injected with some foreign soluble protein. Still others use the term to describe both the sensitized state and the symptoms of intoxication which result from the second injection of the alien protein.

**Active Anaphylaxis.**—The fundamental guinea-pig experiment in anaphylaxis will make the above statements clearer. If a normal guinea-pig is injected with a small quantity of normal horse serum subcutaneously, intraperitoneally, or intravenously the animal hardly shows any discomfort during the injection or at any time afterward, and is in no way distinguishable from its normal mates. The first injection thus causes no obvious ill effect and has apparently produced no harm, and yet profound alterations have taken place which only appear, however, under specific conditions. If this treated or sensitized animal is re-injected with the same horse serum after the lapse of several weeks it now responds with striking symptoms and signs, and may even succumb. The horse serum, which was apparently harmless on first injection, has now acted like a violent poison when injected for the second time. Qualitatively different, but just as marked, symptoms may also be observed in properly prepared rabbits and dogs when the same protein is injected for the second time. This transformation of a harmless substance into a violent “poison” can, however, be observed only if a proper time interval separates the two injections. If the injection is repeated after three or four days, no immediate ill effects are observable, the animal behaves like a normal individual.

We therefore can distinguish three steps in the production of anaphylaxis: (1) sensitization; (2) incubation; and (3) intoxication. These steps may now be considered in more detail.

**Sensitization**

**Sensitizing Substances.**—All the substances which have been shown to sensitize an animal belong to the protein group. It may be said that any soluble foreign protein, of animal or plant origin, may sensitize if it reaches the circulating juices of an animal in an unaltered native state, so that the characteristic structure of the protein is preserved.

The following list, quoted from Doerr (38), will illustrate the variety of substances which have been tested:

I. Animal proteins in solution:
   (1) Foreign serum and its derivatives produced by salting out, by heating, by iodizing, etc.
   (2) Hemoglobins.
   (3) Milk (casein, lactoglobulin, lactalbumin).

1That important changes in the liver occur after simple sensitization has been noted in the guinea-pig by Hashimoto and Pick; these changes, however, bear no direct relationship to the anaphylactic reaction.
40 THE FUNCTIONAL ANALYSIS OF ANAPHYLAXIS

(4) Egg-albumen (ovovitellin, ovomucoid, ovalbumin).
(5) Extracts of organs, tumors, mummies, or of entire animals like oysters, mussels, trout, insects, tenia.
(6) Sweat, bile, albuminous urine, gastric juice, expired air of human beings.
(7) Fluid contents of echinococcus cysts.
(8) Snake venoms.
(9) Ferment solutions containing proteins: papain, rennin, papayotin, pancreatic juice, trypsin.
(10) Nucleoproteins from organs.
II. Cellular animal proteins:
(1) Red blood corpuscles.
(2) Leukocytes.
(3) Spermatozoas, ova.
(4) Syncytial cells.
(5) Cells of organs and tumors.
III. Vegetable proteins in solution:
(1) Extracts of bacteria, yeast and other fungi.
(2) Bacterial nucleoproteins.
(3) Albuminous extracts of seeds.
(4) Purified or pure vegetable proteins like excelsin, gliadin, hordein, zein, viginin, etc.
(5) Crude vegetable fats and oils (always containing proteins).
IV. Cellular vegetable proteins:
(1) Living or dead bacteria, yeasts, schizomycetes.
(2) Pollen granules.

Dosage. Animals.—Exceedingly minute quantities of a foreign protein suffice to induce sensitization. Rosenau and Anderson (107) obtained in one instance sensitization in a guinea-pig with 0.000,001 c. c. of horse serum; and Wells (140) showed that crystallized egg-albumen in a dose of 0.000,000,05 gm. could still render a guinea-pig susceptible. Such infinitesimal quantities, far beyond the range of any balance, or test tube reaction, are not, however, the most favorable doses for the production of a constant and high grade of sensitiveness. General experience has shown that larger doses are necessary in order to obtain marked symptoms on reinjection. The doses vary with the animal species employed, for these show considerable differences in the ease with which sensitization is secured. The most susceptible laboratory animal is the guinea-pig, and a single injection of alien serum varying from 0.01 to 1.0 c. c. sensitizes it so highly that the animal usually dies when the second injection is given intravenously.

Rabbits are not so easily prepared, nor can a high degree of sensitization be obtained as readily and as certainly as in guinea-pigs. A modifica-
tion of the procedure introduced by Arthus (2) probably gives the best results. Arthus injected his rabbits repeatedly (4 to 8 times) with 5 to 10 c. c. of foreign serum; the injections were separated by intervals of 4 to 8 days, and were usually given subcutaneously; sometimes, however, also intraperitoneally. Such rabbits apparently always showed some noticeable reaction when re-injected, and a certain percentage died acutely. Excellent results may be obtained if a not too small series of young rabbits is injected repeatedly at about 5-day intervals with 2 to 3 c. c. of horse serum. The injections are given subcutaneously, intraperitoneally, and intravenously in turn, so that each rabbit receives serum by all three routes. The injections should not be less than four in number. During the process the rabbits require watchful care, as otherwise a number of them are certain to die of respiratory diseases.

There are other methods for the preparation of rabbits reported by Friedemann (53), Friedberger (47), and Scott (121), not reported here, for apparently no method will invariably give a high degree of sensitization in all rabbits. There are always animals which give but a slight or no reaction when the test is made. For this reason it is best to prepare not less than twelve animals; in such a series all gradations of the anaphylactic reaction will probably be obtained on re-injection.

Dogs may be readily sensitized by a single subcutaneous injection of 3 to 5 c. c. of foreign serum. (Biedl and Kraus, 19.) Arthus (2) injected 10 c. c. serum 6 to 8 times at 7-day intervals. A subcutaneous injection of 10 c. c. alien serum in two places, each receiving 5 c. c., yields an excellent sensitization as a rule.

The guinea-pig, rabbit, and dog are the animals whose reactions have been studied most carefully, but they are not the only animals which can be sensitized. References in the literature indicate that horses, goats, sheep, pigs, rats, white mice, pigeons, chickens, geese, ducks and frogs are sensitzizable. That man is sensitzizable is clearly shown by v. Pirquet and Schick's studies. It seems quite probable that practically every species of animal will be found sensitzizable.

Methods.—While the chief, because the most certain, method of producing experimental sensitization is the injection of the foreign protein either subcutaneously, intraperitoneally, or intravenously, there are other procedures of bringing about this result which are of great theoretical importance. Thus sensitization may be inherited, for the susceptibility to a foreign protein is transmitted from the mother guinea-pig to her young, as Rosenau and Anderson (107) showed in their first publication in 1906. Sensitization may be established by feeding guinea-pigs dried horse serum, and dried or fresh horse flesh (Rosenau and Anderson, 107); or by feeding raw cow's milk (Kleinschmidt); or perhaps even by the instillation of one drop of normal horse serum into the intact conjunctival sac (Rosenau and Anderson, 108), though this has not been corroborated by Colombo.
Inhalation of serum produces a specific sensitization, according to Busson, Rosenau and Amoss, Friedberger, and others. Inunction of horse serum-lanolin salves into the intact or scarified skin of guinea-pigs produces sensitization, according to Clough (30). The same author also sensitized guinea-pigs by repeatedly injecting mixtures of horse serum and gum arabic into the vagina or rectum of guinea-pigs. That sensitization may be accomplished by these procedures is of value in explaining those cases in the human being where the first injection of an antitoxin produces a more or less severe anaphylactic reaction. The experimental proof that sensitization may be inherited or brought about without any injury of the mucous or serous membranes or the skin is of great value in the endeavor to explain certain so-called idiosyncrasies of man.

Specificity.—When an animal has been sensitized with a certain foreign protein, horse serum for example, a reaction is only obtained when the animal is reinjected with horse serum; the injection of rabbit or goat serum is without effect. (Otto, 89; Rosenau and Anderson, 108.)

This specificity of the reaction is outspoken and sharp when proteins of widely different species are chosen, but there are group reactions when proteins of closely related species are employed. Thus Rosenau and Anderson (108) report that guinea-pigs sensitized with hen egg-white react to a subsequent injection of duck egg-white or vice versa. The anaphylactic reaction is therefore specific in the same sense that hemolysins, agglutinins, and precipitins are specific. These group reactions have been especially studied by Wells and Osborne (142). These investigators used the purest plant proteins ever employed in their experiments. They found, for example, that guinea-pig sensitized with gliadin from wheat or rye give a strong anaphylactic reaction with hordein from barley, but this reaction is not as marked as if the homologous protein had been employed. Similar results are obtained if the sensitizing protein is hordein and the second injection is gliadin. As these two substances are chemically distinct though similar proteins, Wells and Osborne believe that the specificity of the reaction is determined by the chemical constitution of the protein rather than by its biological origin.

There is another form of specificity which must be briefly touched. In 1904 Wolff-Eisner (135) found that sensitization may be produced by organs. This organ specificity is especially pronounced in the crystalline lens of the eye. Uhlenhuth (122) established that lens protein produces precipitins which act specifically upon the lens proteins of all animals, and not only upon the special lens protein used for the production of the precipitin; also that these precipitins affect no other protein. For the anaphylactic reaction Kraus, Doerr and Sohme, among others, demonstrated that rabbits sensitized with bovine serum do not react to bovine lens extracts, and that rabbits prepared with bovine lens extracts react only to lens extract, but not to bovine serum. This biological differen-
tiation may be great enough that guinea-pigs can be sensitized with the crystalline lens of their own eye and the anaphylactic reaction obtained later by injecting an extract of the lens of the other eye. (Uhlenhuth and Handel. See also Romer and Gebb and Kapsenberg.)

Another clear example of organ specificity is shown by the behavior of blood serum and red blood corpuscles of the same animal. Guinea-pigs prepared with serum are only slightly, or not at all, sensitized to the homologous red blood corpuscles, and vice versa (Thomsen).

Investigations with liver, kidney, spleen, thymus, and brain tissue, also indicate that their proteins differ from that of the serum, and are capable of sensitizing an animal. There are, however, observations which show that a serum used for sensitization and intoxication may give active cross reactions with organ proteins. (Pearce, Karsner, and Eisenbrey, 91.)

Regarding the organ specificity of the placenta, the statements in the literature directly contradict one another. Some affirm and others deny that sensitization and subsequent intoxication of an animal can be effected by extracts of placental tissue from the same species. A similar condition prevails with regard to fetal serum.

Influence of Various Manipulations Upon the Sensitizing Substances.

—That the sensitizing property of protein is very resistant has been demonstrated by Rosenau and Anderson (107, 108, 109), and by Wells (132), among others. Drying and redissolving, heating to 60° C. for 6 hours, precipitation by ammonium sulphate and dialysis, or the addition of iodin, had no effect on the sensitizing property of horse serum. The sensitizing property disappears almost entirely, however, when horse serum is heated to 100° C. for one hour. Peptic and trypptic digestion destroys the sensitizing power but slowly, and sensitization may still be obtained with solutions which show no coagulable albumin. Cleavage products of the proteins, however, do not in general sensitize; even the change of crystallized egg-albumen into acid albumin weakens, and the change into alkali-albuminate destroys the sensitizing property entirely.

Incubation

The period of incubation is the time interval which elapses before the injected animal shows symptoms when re.injected with the same protein. It represents the length of time which the body requires for so altering certain reactions that the reinjection of the formerly harmless, or nearly harmless, protein now acts like a violent poison. If the reinjection is given too early no effect is obtained, and the animal behaves like a normal individual. This condition of sensitiveness develops gradually, reaches a maximum, and then diminishes again in some species, while remaining more or less constant in others.

The duration of the period of incubation before sensitization is estab-
lished depends largely upon the animal species and the method of test, and to a much less extent upon the quantity of soluble foreign protein injected for the first time, or the site of injection.

Ranged in order of sensitiveness we have: (1) guinea-pig; (2) man (Doerr); (3) rabbit; (4) dog. In the guinea-pig the period of incubation is about 10 days (Rosenau and Anderson, Otto); in man 7 to 12 days (v. Pirquet and Schick); in the rabbit 8 to 15 days after the last injection (Arthus); in the dog 2 to 4 weeks (Biedl and Kraus).

Small doses of the protein, less than 0.0001 c. c. horse serum, delay the development of sensitization, and large doses, over 10 c. c. horse serum, appear to exert the same effect. Heating the protein to 80° C., or any method which partly denatures it, delays the onset of sensitization.

The site of injection exerts some influence, but it amounts to only a few days' difference. If medium doses are employed for sensitization the periods of time given above will be found fairly accurate.

After sensitization has been developed this state may continue for a greater or less period of time. Anderson and Rosenau (110) report that guinea-pigs sensitized with a single injection of horse serum remain sensitive during life, which is about 3. years; the degree of sensitization, however, is considerably decreased after 3 years, so that 5-10 times the original lethal dose is then merely toxic and does not kill (Auer, 9). In human beings typical anaphylactic symptoms have occurred when serum was reinjected about five years and longer after the first injection. (Currie, Goodale, Darling, 35.) In the rabbit acute death may still be obtained four to six weeks and longer after the last sensitizing dose (Arthus' method). Scott (121), however, reports that sensitization disappears in the rabbit soon after the twentieth day. In the dog also sensitization may persist for weeks and months, after a single sensitizing dose of horse serum; in one surviving dog Auer (9) obtained the typical blood pressure effect one year after sensitization.

INTOXICATION

When a sensitized animal is reinjected with the same sensitizing protein various functional disturbances occur which did not appear when the substance was first injected. These disturbances, while they show certain resemblances in the different animal species, disclose marked differences in the way the symptoms are combined and in the functional alteration which predominates. The symptoms vary with the method demonstrating the state of sensitiveness, and they vary also according to the degree of sensitization the tested animal has attained or retained.

The most obvious symptoms and anatomical changes which occur during the anaphylactic state, both acute and subacute, are fairly constant for each species with a given procedure for reinjection of the protein. This picture does not vary with the nature of the proteins employed, but all
proteins, irrespective of their chemical nature and derivation, cause the same anaphylactic alteration in the same species. Anaphylaxis produced by horse serum is identical with that produced by edestin, a protein from hemp seed. There is but one perhaps complex anaphylactic picture for each species, but it may be developed by a large number of different substances, which, however, all belong to the protein group.

**Symptoms.**—In general it may be said that respiratory disturbances characterize the acute anaphylactic intoxication in the guinea-pig; circulatory changes in the rabbit; gastro-intestinal and circulatory alterations are most prominent in the dog, while man shows marked skin lesions in the majority of cases, though respiratory and circulatory changes also occur. A fairly detailed description of the symptoms and their analysis will be given later.

**Method.**—**Dosage.**—The intoxicating dose of the protein may be administered in the same variety of ways with which sensitization is produced. The main methods are by subcutaneous, intraperitoneal, and intravenous injection. For quantitative work where it is necessary that differences in the rate of absorption be avoided, the intravenous route is imperative. The vein chosen in guinea-pigs is the external jugular; in the rabbit the lateral ear vein or the jugular; and in the dog the saphenous or the jugular vein. The intracardiac method is not to be recommended as a substitute for the intravenous injection; it is easier to attempt in the guinea-pig, but serious damage to the heart is by no means rare. Intoxication may be caused by subdural, intracerebral, and intraspinal injections; by inhalation, or by injection of the protein intratracheally.

While the quantity of foreign serum necessary to produce symptoms on reinjection varies with the site of injection, and with the criteria adopted, it is much larger than the amount which sensitizes. In the guinea-pig, for example, which has been sensitized with horse serum it is probably impossible to give a dose subcutaneously which kills with certainty. Lewis (76) states that 5 to 6 c. c. subcutaneously always give a well-marked reaction, so that 15 to 20 c. c., if absorbed at the same rate, would be fatal. When the reinjection is given intraperitoneally 5 to 6 c. c. kill; and the fatal dose is still smaller with intravenous reinjection. To kill highly sensitized guinea-pigs 0.01 to 0.02 c. c. is sufficient. Both in the rabbit and dog acute exitus cannot be obtained when the foreign protein is injected subcutaneously, due undoubtedly to the fact that the protein is absorbed too slowly, and thus never reaches a sufficiently high concentration in the blood. Figures given by Doerr and Russ show this difference between the amount of the sensitizing and intoxicating doses; they furnished evidence that the minimal sensitizing dose in the guinea-pig is 200 to 2,000 times smaller than the quantity of the same protein which causes symptoms or acute death when injected intravenously.

**Sensitizing and Intoxicating Substances of the Foreign Protein.**—
Largely through the work of Rosenau and Anderson, Doerr and Russ, and Wells, it is generally accepted that the sensitizing substance and the substance producing the anaphylactic symptoms are identical. The evidence, however, is not absolutely conclusive, although it seems certain that the protein molecule as a whole exerts both of these functions. The work of Vaughan and his collaborators, for example, indicates that all true proteins can be split into a toxic and a non-toxic fraction by heating the protein a few hours at 78°C in a 2 per cent. solution of sodium hydrate in absolute alcohol. The toxic fraction kills guinea-pigs with symptoms which resemble those observable in anaphylactic animal, but it cannot sensitize. The non-toxic fraction, however, usually sensitizes, but the sensitization is specific only for the entire protein molecule, and not for the non-toxic portion itself. These experiments show that a separation of the sensitizing and intoxicating principles is apparently possible.

THE ANAPHYLACTIC REACTION

General Symptoms.—Guinea-Pig.—The symptoms obtained when a sensitized animal is reinjected with the same protein vary considerably in the different species, though the difference on analysis is largely a quantitative one. After a sensitized guinea-pig has received an intravenous injection of the foreign protein, and is then liberated, the animal remains quiet for about a minute, when restlessness appears. It moves about, the hairs bristle over the neck, head, and body; it sneezes frequently and sits up on its hind legs to rub its nose vigorously; occasionally the animal jumps suddenly. Within two or three minutes the animal is unable to stand, falls on its side, and violent tonic and clonic convulsions develop. In the intervals between convulsions the animal lies motionless on its side; the legs are neither spastic nor flaccid, and a pinch of the toes usually elicits a vigorous kick. Respiration during this stage is slow and labored, and may cease for a short time. The final stage is ushered in by a group of respirations, which swiftly get weaker, and finally stop entirely. The entire process need last no longer than three minutes. The heart on palpation usually beats vigorously and regularly, though slowly, and continues to beat for some minutes after all respiration has entirely stopped.

This picture is completed if another experiment is made with a guinea-pig stretched out on its back. After recovery from the ether anesthesia employed to introduce a cannula into the jugular vein, the toxic or second injection is given through this cannula, and the cannula then washed clear by 1 or 2 c. c. of saline or Ringer solution. Within thirty seconds the respirations quicken noticeably for a short time, and the animal struggles and squeaks shrilly. Careful inspection now shows that the
respiration is slower, and that the thoracic wall, especially the costal margins, sinks in with each inspiration. This sinking of the chest wall increases more and more, and the respirations become still slower, but much more powerful and labored. At this stage, especially in young animals, one may see that the lower part of the sternum and costal margins are drawn inward to an astonishing degree with each inspiration. Now tonic and clonic convulsions develop, accompanied by no sound, or, at most, by a choking, feeble squeak; the pupils dilate; the mucous membranes of the mouth appear bluish; there is often a spurt of urine, and a number of fecal pellets are passed. The convulsions cease after a short time, and the animal lies motionless without any respiration, but the heart is seen beating with strong, slow, regular pulses; the chest looks fuller than normal; and there may be peristalsis, which is easily visible through the relaxed abdominal walls. After a respiratory stoppage of about one minute, which may be broken by an occasional inspiration followed by a convulsive active expiration, a group of respirations appears. This terminal group is formed by respirations which are at first slow and of fair strength, but rapidly become swifter and weaker, and finally disappear about one minute after their onset. Each of these terminal respirations is preceded by a dilatation of the nostrils and an opening of the mouth, which is maximal at first; as the respirations weaken the opening of the mouth and the dilatation of the nostrils decrease, and they also disappear. The order of stoppage is first the respiration, then the opening of the mouth, and finally the inspiratory widening of the nostrils. Cessation of respiration is now permanent. At this time the heart still beats regularly and strongly, though apparently at a slower rate than during the respiratory stoppage, and its beating usually continues for many minutes after respiration has permanently ceased.

The striking symptoms just described for the guinea-pig appear when the injected animals are highly sensitized and when a lethal dose is given intravenously. If the test animal is not highly sensitive, or if the dose injected is sublethal, the picture may show all gradations from the fatal type described to mild effects chiefly characterized by restlessness, sneezing (coughing?), erection of the hair, moderate bucking movements; and discharge of feces and urine. It is interesting and instructive that animals which show a most severe reaction may nevertheless apparently fully recover after an hour or two, and feel well enough to fight with their neighbors. On the other hand, there is also a protracted course of the intoxication¹ which leads to death after some hours. In these animals paralytic symptoms are the most noticeable because the most lasting.

RABBIT.—In this animal the anaphylactic reaction reveals itself either

¹The word intoxication is used merely as a descriptive term and does not postulate the existence of a true toxin or toxins as the cause of the anaphylactic reaction.
as a local or a general manifestation, depending upon the method of rein-
jection of the foreign protein.

The general reaction is obtained when a highly sensitized rabbit is
reinjected intravenously with the foreign protein. The respiration quick-
eness at first and the animal lies down upon its belly for a time, often with
the hind legs stretched out; a greater or smaller number of dry normal
fecal pellets are passed. Within a few minutes, however, the respiration
slows, and the animal suddenly falls over on its side with clonic convul-
sions of short duration. The head is retracted strongly, the iris vessels
(easily seen in white rabbits), gums, and tongue are pale, the pupils are
wide. The convulsions are sometimes preceded or accompanied by a few
feeble cries. After the convulsion the animal lies motionless without
respiration, and immediate palpation of the chest, as a rule, fails to detect
any cardiac pulsation. After less than one minute the terminal group of
gradually weakening respirations appears; as in the guinea-pig, these are
preceded and accompanied by an opening of the mouth. The animal now
shows no visible or palpable heart beats or respirations; it is perfectly
relaxed, and the abdominal walls bulge when the animal is placed on its
back. As a rule, one sees now very active peristalsis and antiperistalsis
of the cecum, which is sharply outlined by the relaxed abdominal parietes.
The time interval between injection and terminal group of respiration
need not exceed three minutes. It is deserving of notice that the respira-
tion of the rabbit during this reaction is never dyspneic.

A reaction of this fulminant character cannot be obtained with the
same certainty in the rabbit as in the guinea-pig. In the latter animal over
95 per cent. of a series prepared and reinjected with an adequate dose will
succumb; in the rabbit, however, only a number of a series prepared in
exactly the same way will succumb acutely as described above. The others
show on inspection rapid respiration without group formation, as in the
normal rabbit, more or less marked discharge of normal scybala, often
erception of the hair of the body, a temporary but well-marked miosis of
the pupil, usually lasting for some minutes. During the stage of polypnea
the animals lie quietly on their belly with the head moderately retracted
and the hind legs often extended. Within half an hour or less after the
reinjection the animal may seem perfectly normal.

In animals which recover from an intravenous reinjection Arthus (2)
describes a gradually developing cachexia, accompanied by a diminution
in the number of red corpuscles and a lowered hemoglobin content. This
cachexia leads to death in a few weeks. Such a cachexia does not develop
in guinea-pigs which survive an intravenous injection; they have been
found in excellent condition over a year after a very severe reaction.

The local reaction appears when a sensitized rabbit is reinjected sub-
cutaneously, and constitutes the well-known phenomenon of Arthus. Ar-
thus (2) describes the process as follows: If the rabbit is sensitized by
the subcutaneous injection of 5 c. c. of horse serum every six days the first few injections will be absorbed in a number of hours. The fourth injection usually produces a soft infiltration which is not absorbed before two or three days. The fifth injection causes an edematous infiltration which is harder, and is not absorbed before five or six days. The sixth injection rapidly produces a white, solid, compact, subcutaneous mass which is not pus, and which may persist for weeks. Similar but more pronounced changes are obtained on the seventh injection of 5 c. c. of horse serum; the skin over the subcutaneous mass becomes red, then pale, and begins to dry, and a spot of gangrene develops which produces a refractory wound. The general condition of the animal, however, remains excellent.

These local phenomena are not due to the repeated injection of the foreign protein into the same locality, because they are also obtained when each subcutaneous injection is given in a different place, or when all injections except the last have been intraperitoneal. This last injection, however, must be given beneath the ventral or thoracic skin if the typical phenomena are to be produced; injection beneath the skin of the ear, for example, produces only a voluminous edema, according to Arthus. The quantity of protein, horse serum in Arthus' experiments, played no apparent rôle; less than 1 c. c. at each injection produced the same tissue changes as 10 c. c.

A similar local reaction (phenomenon of Arthus) may also be obtained in the guinea-pig (Lewis) if the animals do not die before its development. In the dog Arthus was unable to obtain this local reaction; after seven subcutaneous injections of horse serum at 7-day intervals the last dose was entirely absorbed within four to five hours, and no change was observable during the next three days at the site of injection.

Doe.—This animal, when not anesthetized, also exhibits striking symptoms, chiefly gastro-intestinal, during the anaphylactic reaction. The following description is based largely upon the descriptions of Biedl and Kraus (19) and Richet (101). If a sensitized dog is reinjected intravenously with the same protein used for sensitization the animal shows a marked excitement within one minute, often before the injection is finished. The stage of excitation does not last long, and the animal begins to make swallowing motions. Soon retching develops, followed by vomiting. The vomitus, according to Richet, may be bile-stained, bloody, or even fecal, and vomiting occurs even though the animal is fasting.

While vomiting the animal is usually able to stand, but nevertheless exhibits the usual marked muscular weakness. Associated with the vomiting, which occurs repeatedly, there may be fecal discharges. The animal now usually lies or, rather, falls down, and remains quietly in the same position, breathing without difficulty. At no stage is there any noticeable dyspnea. The dog may now die or slowly recover within the next few hours. According to Biedl and Kraus, the corneal reflex is
always present, and the animals react to stimulation of the skin, even during the stage of deepest depression.

Acute death within thirty minutes after reinjection does not occur as frequently as in the guinea-pig, but nevertheless it is not infrequently obtained, provided that the sensitization has been produced with fairly massive doses (10 c. c. horse serum, for example), and that the reinjection is not given before at least four weeks have passed. (See Fig. 7, page 71.)

Man.—The human subject also shows well-marked anaphylactic reactions, which are chiefly manifested in the symptom-complex called serum disease by v. Pirquet and Schick. In this group there are remarkable disturbances characterized by their occurrence after injection of some therapeutic serum which is usually obtained from the horse. These manifestations are general swelling of the lymph glands, skin eruptions of apparently inexhaustible variety, remittent fever, edema of the face, and later of the dependent parts of the body, severe pains in the metacarpophalangeal, wrist, and knee joints, without objective changes, and leukopenia. The time of onset or period of incubation of the serum disease varies with the number of the injection; in reinjected individuals the period is much shorter than in those receiving the therapeutic serum for the first time. The percentage of incidence of the disease varies definitely with the quantity of the serum injected. A more detailed description of this interesting complex will be given later.

The serum disease is, however, not the only group of pathological changes evoked by the foreign serum in sensitized man. Severe effects which threatened life, and even deaths, have been reported from the therapeutic injection of sera. The symptoms described indicate a sudden and remarkably severe effect upon the respiratory and cardiovascular systems, effects which especially characterize the anaphylactic reaction in the guinea-pig, rabbit, and dog. Reactions of this character have been obtained in man with small doses of serum, not more than 1 c. c. in certain cases, and moreover after subcutaneous injection where absorption is necessarily slow. Doerr, therefore, grades man between the guinea-pig and rabbit in sensitiveness.

Other Animals.—Though all the fundamental information we possess about anaphylaxis has been gained from the study of the animals mentioned, the reaction has been sought for in many other species. The results, however, do not yet warrant detailed consideration because little was accomplished beyond the demonstration that anaphylaxis occurs. The establishment of this fact is, of course, important, but otherwise the experimental yield was small. This result was largely due to the attitude of the investigators; most of them sought apparently only for those functional and anatomical changes which became obvious after they had once been pointed out. Such a viewpoint, however, is not one which will increase our knowledge of the fundamental alterations which a new disease produces,
for these alterations may differ considerably in the different species of animal due to their adaptation to special needs, although their systems of organs are qualitatively alike. A change which is profound, and even fatal, in one species may only be indicated in another and, indeed, might escape detection. For this reason it is necessary to study each species for itself, and while the investigator should be alert to note resemblances of reaction in the various species, he should be still more alert to discover new types of reaction. Comparative investigation of this character would give a rounded picture of the effects which the same process may induce. This is of special importance because man seems to have the capacity of reacting in many different ways to the anaphylactic intoxication, and at least some of these human forms of reaction seem very similar, and may even be identical with those observable in various animals. As it is a priori probable that all the anaphylactic reactions occurring in man will show their analogue, if not homologue, in one or the other of the lower animals, the scientific and practical value of a comparative study of the phenomena in question is apparent, for a rigid experimental investigation, devised to answer specific questions, cannot and must not be carried out in man.

THE EXPERIMENTAL ANALYSIS OF THE ANAPHYLACTIC REACTION

The anaphylactic reaction expresses itself by a disturbance in the function of numerous organs, and these disturbances may be more or less obvious on mere inspection. A closer insight into their mechanism, however, has only been obtained after the anaphylactic complex was analyzed from the viewpoint of modern experimental medicine, that is, when the ordinary procedures of physiology, pharmacology, and chemistry were brought to bear upon the problem.

It must be emphasized again that the anaphylactic disturbances are the same, no matter what foreign soluble protein is used to produce them. In the following pages an experimental analysis of the anaphylactic symptom picture in the guinea-pig, rabbit, dog, and man will be given. What the main symptoms are has already been briefly indicated.

Respiratory System.—Anaphylactic changes in the respiration are shown by the guinea-pig in an exquisite fashion when the protein is reinfected intravenously, and mere inspection clearly indicates this. The character of this involvement was not, however, realized until Auer and Lewis (10) demonstrated that acute death in the anaphylactic guinea-pig was due primarily to an asphyxia brought on by a swiftly developing stenosis of the bronchioles, and that this stenosis exhibited itself by a striking macroscopic alteration of the lung which could serve with proper precautions as an easy index of the anaphylactic reaction in the guinea-pigs. Evidence for these facts was brought out in a variety of ways. The guinea-pig was
allowed to breathe from an air container connected with a Marey tambour, which not only registered roughly quantitatively how much air entered and left the air receptacle at each respiratory cycle, but also showed the character of each respiratory cycle, whether the air entered or left the lung promptly or slowly. At the same time the intrapleural pressure was recorded by means of a Meltzer pleural cannula. About half a minute after injection of the foreign protein into guinea-pigs prepared in this manner it was noted that inspiration and expiration recorded by the tambour in connection with the air receptacle showed a marked decrease in amplitude, and were of longer duration than before, as was indicated by the sloping course of the lever during its inspiratory descent and expiratory ascent. The intrapleural pressure changes corresponding to these respirations were greater than normal, showing that the animal was experiencing difficulty in getting air into and out of the lungs. After a few seconds the records showed that no air was entering or leaving the air receptacle, although the intrapleural pressure changes (due to the action of the respiratory muscles) were enormous. The action of the respiratory muscles was apparently unimpaired at this stage, and yet their tremendous efforts were entirely unavailing to cause any air to enter or leave the lungs; even the violent convulsions which now appeared had no effect upon the volume of the lungs, for the lever of the tambour connected with the air vessel traced a straight line which was near the inspiratory level of the tracing. This experiment showed clearly that the nervous and muscular mechanism of respiration showed little, if any, impairment, while the lungs were apparently the seat of some profound change which prevented the entrance and exit of air.

Experiments were then carried out with guinea-pigs which had been curarized, whose vagi had been cut, or whose spinal cord, medulla, and basal brain had been destroyed by pithing. Artificial respiration was, of course, necessary under these conditions to maintain life. When the intrapleural pressure of such animals was recorded the tracings gave valuable information. Shortly after injection of the toxic dose the tracing, which records the fluctuations of intrapleural pressure brought on by the constant volumes of air forced rhythmically into the lungs through the trachea, shows remarkable changes. Immediately after the injection the excursions of the lever decrease moderately in amplitude, then they increase in amplitude, and finally they decrease rapidly to such a degree that the lever does not record any respiratory fluctuations at all, though the machine delivers the air at the same rate, pressure, and volume as before. The lever comes to rest, as far as respiratory oscillations are concerned, at various points between the expiratory and inspiratory levels of the tracing, never, however, in a typical experiment at the expiratory level. The lever records now only the volume changes of the heart. (See Fig. 1.) Similar tracings were obtained when a lobe of a sensitized guinea-pig's lung
FIG. 1.—VOLUME CHANGES OF THE LUNG IN A GUINEA-PIG DURING ACUTE ANAPHYLAXIS. The tracing was obtained from a pithed guinea-pig by means of a pleural cannula connected with a Marey tambour. Upstroke of the recording lever means inflation; downstroke, collapse of the lung (artificial respiration throughout the experiment).

The animal was sensitised by 3 mg. of edestin dissolved in 1/20 NaOH injected subcutaneously. After about 6 weeks 0.5 mg. of edestin in 1/20 NaOH was injected into a jugular vein (first broad white band in the tracing; the second white band below the time line, which records 4 second intervals, shows the injection of 1 c.c. saline solution to wash out the cannula).

Note the initial decrease in the amplitudes after the injection of serum (bronchoconstrictor effect); then the increased amplitudes (bronchodilatation) and finally the abrupt abolition of all pulmonary oscillations, although the artificial respiration machine delivers the same amount of air as before (extreme bronchoconstrictor effect). Now only the volume changes of the heart are recorded; note the abrupt changes in heart rate: cardiac block.
was placed in an oncometer and its volumetric oscillations with artificial respiration recorded before and after injections of the toxic dose of protein.

These experiments show definitely and unmistakably that the second injection causes by peripheral action in the lung (the central nervous system being excluded by pithing) a stenosis in the air passages, which becomes so extreme that the respiration machine cannot force in air; the complete stenosis being preceded by a period of increased ease of entry of the air; and this, in turn, being preceded by a period of slightly decreased ease of entry, shown by fluctuations in the amplitude of the lever which records the volume of the lungs or the intrapleural pressure. The records also show that the final volume of the lungs must be greater than the normal expiratory volume of the organ, for the lever comes to rest at a higher point than the expiratory level. Figure 1 illustrates these changes.

A condition of such extreme stenosis of the air passages that the most violent inspiratory and expiratory efforts of the animal, or the blast of a respiration machine, cause no change in the volume of the lung must obviously bring about asphyxia. Hardly any other proof is necessary, but additional evidence is easily brought forward. If the blood pressure is recorded in an anaphylactic guinea-pig it will be noticed that within one minute after the reinjection the blood in the cannula turns very dark, even black; the mucous membrane of the mouth become bluish, the pupils dilate widely, and violent convulsions appear. If a sample of blood is now taken from an artery it looks almost black, but becomes bright red when diluted with a little salt solution and shaken a few times. Though no gas analyses have been made of the blood, it seems quite certain that carbon dioxide is present in large amount. Cardiac failure is not the cause of this asphyxia, because the heart keeps on beating regularly and powerfully for many minutes after all respiration has definitely ceased. If on autopsy the root vessels of the heart are compressed by a dissecting forceps, the organ excised, and the forceps released, the systole of the heart drives the black blood in the left ventricle many inches into the air. Failure or weakness of the cardiac pump cannot thus play an important rôle in the production of this high grade of asphyxia.

It was stated before that the evidence indicated that the volume of the lung after acute anaphylactic death is greater than that of the lung at the time of a normal expiration. The autopsy of any guinea-pig which dies acutely (3 to 10 minutes) from the reinjection gives full support to this inference and furnishes the anatomical evidence for the functional respiratory alterations which have been described. Auer and Lewis describe the lung picture as follows: On opening the chest the lungs present a striking sight; the lungs do not collapse, as normal lungs do when the thoracic cavity is opened, but remain almost fully distended. They look pale bluish-pink, and apparently form a cast of the thoracic cavity; even when
excised in toto there is practically no collapse, and the posterior surfaces of the lung often clearly show the markings of the ribs. The excised lungs are light, soft, and spongy, and float on water like a cork. Pieces of lung tissue cut off do not collapse, but remain distended; the surface of the cuts is usually dry, and on pressure a good amount of air can be expressed. Occasionally this pressure reveals some small foci of white foam, as if there were beginning pulmonary edema; occasionally small hemorrhages

**Fig. 2.—Anaphylactic Lung in the Guinea-pig, with and without Atropin.** The figure on the left shows a practically normal collapsed lung. The figure on the right illustrates the typical anaphylactic lung. Both guinea-pigs were sensitised by subcutaneous injection of 1 c.c. horse serum. After 55 days the right vagus of each was resected in the neck. After 68 days from the date of sensitisation both received 0.3 c.c. of a 10 per cent solution of heated horse serum intravenously. One animal was previously given 3 mg. of atropin sulphate subcutaneously. The atropin animal exhibited but slight symptoms of anaphylaxis and was killed later by severing the medulla. The lungs collapsed in a normal manner on opening the chest except the right upper lobe. The second guinea-pig, however, died within 5 minutes of the injection of serum and its lungs showed the typical anaphylactic fixation in a full, inspiratory position. The pictures also show that the denervation of one side of the lung exerts no effect on the anaphylactic reaction, or upon the action of atropin.

were seen on the surface of the lungs. The trachea and bronchi usually were dry, but often showed a marked congestion of the mucosa.

Figure 2 illustrates this remarkable lung condition, which was first noted, but only casually described, by Gay and Southard (56), although these authors definitely state that they were “inclined to regard this emphysema as the effective cause of death in the quickly fatal cases.”

The causation of this interesting anatomical change in the lung was
attributed by Gay and Southard (56) to an emphysema produced by a diaphragmatic spasm, which is secondary to a stimulation of the medullary and phrenic centers of respiration. Auer and Lewis disproved this theory by showing that the typical lung picture is promptly obtained in guinea-pigs which have been curarized, or whose central nervous system has been destroyed. These authors advanced the view which has been generally accepted that the anaphylactic lung in the guinea-pig is produced by a tetanic contraction of the muscles of the finer bronchioles. Their reasons were briefly as follows: the fluctuations in volume which the anaphylactic lung shows during artificial respiration; their final disappearance, leaving the lung in a fixed inspiratory position even when excised; the absence of collapse of small pieces when cut off, the rich content in air; moreover, the fact that atropin can reestablish the rhythmic expansion and collapse of a typical immobile anaphylactic lung; all these facts indicated that the muscles of the finer bronchioles were at fault, for previous work had established that these structures profoundly affected the function of the lung.

It was well known that stimulation of the peripheral vagus nerve caused contraction of the bronchioles and produced stenosis effects in the lung (Einthoven, Dixon and Brodie), and these effects were apparently identical with those recorded in the anaphylactic guinea-pig; it was also established that blood vascular changes due to this stimulation of the vagi would not account for the lung changes (Dixon and Brodie, 37). Furthermore, since Dreser (41) and others showed that atropin abolished the bronchomotor effect of vagus stimulation, it seemed legitimate to attribute the anaphylactic lung changes in the guinea-pig to a tetanic contraction of the muscles of the finer bronchioles which effectively occluded their lumen so that the contained air was imprisoned and the animal necessarily succumbed to an asphyxia.

This mechanism easily explains how the distended inspiratory state of the lungs is produced and maintained. As the bronchial muscles gradually begin to contract the lungs fail to collapse fully during expiration because the air now leaves with greater difficulty, due to the narrowing air passages. Some air therefore remains in the lungs when the next inspiration occurs. This incoming air meets the same resistance, but nevertheless more air enters the alveoli than leaves them, because each inspiration utilizes the entire available passageway, for the increased negative pressure in the thorax tends toward an opening of the bronchioles. Expiration, on the other hand, and especially active expiration, tends toward narrowing still further the already narrowed tubes by increasing the pressure resting on the outside of these tubes, for the intrathoracic pressure becomes positive during active expiration. Therefore, in spite of the fact that the expiratory efforts of the animal are more powerful than the inspiratory efforts, less air is expelled than taken in, and the lungs must become sooner or later maximally distended. Moreover, this deficient
alveolar ventilation leads to an accumulation of CO₂, and this gas has been shown by Einthoven and by Dixon and Brodie to produce a tonic constriction of the bronchial muscles. This increases the stenosis, and consequently the asphyxia, still more, until no air enters or leaves the lung and the animal succumbs. If the lungs are now excised they will be found in a state of maximal inspiration, which is maintained for hours. (See Fig. 2.)

A beautiful picture of the whole process may be easily obtained by observing the effect of the reinjection in a pithed guinea-pig whose chest has been split transversely. After injection of the toxic dose one may see that the artificial respiration at first produces a greater expansion and collapse of the lungs due to a relaxation of the bronchomotor muscles; very shortly after this the lungs do not collapse fully during expiration, and with each succeeding blast of air the expiratory collapse of the lungs becomes less and the distention more, until after a few minutes the artificial respiration produces no further increase and the expiratory pause no decrease in volume. The lungs are fixed in an immobile, inspiratory position, which is not altered when the organ is excised.

The experimental facts brought forward by Auer and Lewis were soon corroborated in general by a number of observers, especially Anderson and Schultz (1), and Biedl and Kraus (20), and at present no one doubts that acute anaphylactic death in the guinea-pig is caused by an asphyxia which is brought on by the development of a stenosis in the pulmonary air passages of the animal. The only exception is perhaps Richet (101), who is unwilling to accept the interpretation that a tetanic contraction of the bronchioles causes the asphyxia to which the guinea-pig succumbs, because (1) this is not the cause of death in dogs, and it is inconceivable to Richet that anaphylaxis in the guinea-pig and dog is different; (2) artificial respiration does not prevent death; (3) it has not been proved that the blood is asphyctic. The reader will notice that most of the objections urged by Richet have already been partly answered. Auer and Lewis and Biedl and Kraus showed definitely in graphic records (see Fig. 1) that artificial respiration does not save the life of the guinea-pig for the simple reason that the air cannot enter the lung because of the stenosis in the air-passages; even a pressure of such degree that enough air was discharged per blast to satisfy the needs of an adult dog was insufficient to overcome the stenosis (Biedl and Kraus). The same thing is true when the anaphylactic animal breathes spontaneously: after a certain time no air enters or leaves the alveoli; it therefore would be perfectly useless to place animals in an atmosphere of oxygen as Richet (101) suggests, for none of it could enter the alveoli after the anaphylactic reaction was fully under way. The other objection that it is inconceivable for anaphylaxis to be different in the different animals will answer itself in the section dealing with the analysis of the symptoms in the different animals. It may not be amiss in passing to point out that an attitude which a priori demands an identity of reaction to the same causative agents in different animal species necessarily leads to erroneous conclusions.
By further experimentation upon guinea-pig's lungs Auer (5) demonstrated that the typical lung picture could be obtained after the bronchial muscles of one side of the lung had been deprived at least partly of their motor innervation by section of the corresponding vagus in the neck. In a number of series of animals one vagus was resected either before or after sensitization had been established; the reinjection was given after various time intervals. The result showed no definite difference between the two halves of the anaphylactic lung. (See Fig. 2.) As thirty-three days passed in one series between vagus section and the injection of the sensitizing dose, and the second or toxic dose was injected fourteen days later, and as the resultant lungs did not differ from those obtained in guinea-pigs with intact vagi, it is legitimate to assume that the nerve and nerve endings were degenerated, and that the denervated muscle itself responded to the sensitizing and the intoxicating doses. Auer obtained no evidence that the vagus bronchomotor endings played a rôle in the production of the anaphylactic reaction, but does not deny this possibility.

The anatomy and histology of the anaphylactic guinea-pig's lung were extensively studied. Schultz and Jordan (119), in a valuable contribution, proved among other facts that the stenosis of the pulmonary air passages which causes death is localized in the secondary and tertiary bronchi. The tetanic contraction of the muscle coat folds the mucous membrane of this area into a plug which occludes the lumen, and thus brings about asphyxia. The air passages below the level of the secondary and tertiary bronchi were found open, even distended. Schultz and Jordan's studies, made upon stained sections and complete dissections of the bronchial tree of normal and anaphylactic lungs, do not entirely explain the distention of the anaphylactic lung, for small pieces of the lung cut from the periphery of the lobes do not collapse. It is possible that this is due to an increase in the rigidity of the tissue elements. The same authors also note the presence of edema near the bronchial tree. This edema, however, is only rarely extensive, and in the vast majority of experiments with non-toxic sera the lungs show only traces of edema (Biedl and Kraus, 20, 21). If, however, primarily toxic sera are employed, Karsner (71) demonstrated that the guinea-pig's lungs show marked evidences of conglutination of the red corpuscles, hemolysis, hemorrhage, and edema.

The Lungs in Subacute Anaphylaxis.—The macroscopical change in the lungs of the guinea-pig which succumb to acute anaphylaxis are practically not observable when the injected animal dies after the lapse of one-half to several hours. In these delayed cases the lungs usually collapse fairly completely when the thorax is opened. The degree of the collapse observed seems to depend upon the severity of the symptoms and the speed with which death ensues; the sooner death occurs the greater is the distention of the lungs. If guinea-pigs are killed shortly after the main symptoms of a sublethal intravenous injection have passed off, the
lungs always fail to collapse as completely as in a normal animal; one or the other lobe of the lungs, if not all, will always show distention. This demonstrates that the same qualitative change took place in the lungs, though it was not great enough to produce acute exitus. The cause of death in those animals which die subacutely has not yet been established. It is very probable that a number of factors together produce this result, for in these delayed cases extensive hemorrhages are found in the gastrointestinal canal, diaphragm, lungs, heart (Gay and Southard); the vessels of the splanchnic area are congested, indicating a low blood pressure, and the initial asphyxia probably also aids in bringing about death.

Lung Changes in Other Animals.—As acute anaphylactic death caused such a pronounced anatomical and functional change in the lung of the guinea-pig, it was perhaps natural to expect that a similar change would be found in other species of animals. The inference did not prove true, however, at least as far as the dog and rabbit are concerned, and this difference at first produced some confusion among investigators who postulated an identity of the anaphylactic reaction in all animals. In rabbits, for example, which have succumbed acutely to the reinjection the lungs collapse well, but not completely; they look mottled, and occasionally hemorrhages are seen on the surfaces. On closer inspection numerous areas of emphysema are usually visible on the surfaces and borders; the large distended air sacs composing these areas of emphysema are easily visible to the unaided eye. A cut surface may show small areas of fine foam on pressure, as if there were beginning pulmonary edema. The trachea, as in the guinea-pig also, looks bluish and the mucosa is strongly congested. The congestion extends into the pulmonary bronchi. (Auer, 6.) Scott (121) states that the lungs of rabbits retract normally and are rather pale; microscopically he describes and pictures a thickening of the interalveolar septa; the capillaries were compressed and the blood corpuscles seemed peculiarly adherent to the walls. Scott never saw a general edema, though some alveoli contained a little serous exudate, but the lung condition suggested a very early stage of acute edema to him.

In the non-fatal anaphylactic reaction of the dog the lung differs but little, if at all, from that of a normal dog; the lungs collapse well on opening the chest, and show smooth surfaces and borders. There is no indication of any local emphysema such as the rabbit shows, nor are any hemorrhages to be observed on the lung surfaces. There is, however, a functional disturbance, the spasmodic expirations during the stage of excitation which Biedl and Kraus (20) are inclined to interpret as due to a stimulation of the bronchial muscles.

In dogs which succumb acutely the lungs do not collapse completely as a rule, but often remain more or less distended on excision like the anaphylactic lungs of a guinea-pig. They are large, pale, doughy, and pieces which are cut off remain distended and are full of air. There
is no pulmonary edema, nor are hemorrhages detectable on the surfaces of the lungs.

In man the anaphylactic reaction may produce marked respiratory disturbances which are probably identical in their causation with those observable in the guinea-pig. These and other symptoms which have been observed will be considered together in another section of this chapter.

Cardiac System.—Anatomical Changes.—The heart shows a number of anatomical and functional changes during the anaphylactic reaction which have not been extensively studied so far. Gay and Southard (56), in their valuable histological studies of the anaphylactic guinea-pig, were the first to describe cardiac hemorrhages. The hemorrhages are found chiefly on the ventricular surfaces, especially near the apex; the auricles show but few small punctate hemorrhages, which are never extensive, and indeed may be absent entirely, at least on macroscopical examination.

Both in the guinea-pig and the rabbit the production of these cardiac hemorrhages may be directly observed when the thorax is split and the anesthetized animal kept alive by means of artificial respiration. Shortly after the injection of the toxic dose of protein the ventricle, right or left, may show suddenly a dark red spot which often rapidly grows and forms a moderately raised mass during systole of the heart. The hemorrhages may be fairly numerous and discrete; at times, however, they are quite extensive and involve a large part of the ventricular portion of the heart.

These hemorrhages, visible from the pericardial surface of the heart, are especially pronounced in the guinea-pig, and are not obtained to the same degree in rabbits. In the cat subpericardial hemorrhages have been observed by Schultz (115). In dogs hemorrhages visible on the pericardial surface have not been described at all as far as the writer is aware; nevertheless in this animal also gross cardiac hemorrhages occur, but they have not been observed before because comparatively few dogs succumb acutely. The hearts of such dogs often show marked, radially arranged hemorrhages beneath the endocardium, especially on the septal surface of the left ventricle. These hemorrhages in the interior surface of the left ventricle almost invariably involve the left branch of the His bundle (the left branch of Tawara) which forms two main divisions. These branches often show blood-red sections, which may be extensive, where a hemorrhage has occurred into them. In addition there are also hemorrhages into the papillary muscles. The left ventricular cavity shows more extensive hemorrhages than the right. The auricles show but few, if any, hemorrhages, and those are only visible when the auricles are slit open.

Subendocardial hemorrhages of the kind described for the dog are frequently observable in the rabbit and the guinea-pig. (Auer, 9.)

These hemorrhages are not to be explained as the result of violent convulsions during which the general systemic blood pressure is increased, because the hemorrhages are also obtained in curarized or an-
THE ANAPHYLACTIC REACTION

esthetized guinea-pigs, rabbits, and dogs, where the animal remains perfectly quiet. The systemic blood pressure, moreover, seems to play a subsidiary rôle, because, in the dog, the blood pressure is low—40 mm.

approximately—within less than a minute after the reinjection, and yet the endocardial hemorrhages in this animal may be just as extensive as in the rabbit and guinea-pig, where the blood-pressure curve in the fatal cases shows an initial rise and subsequent slow fall. These hemorrhages seem rather to be the result of local constrictions which appear in the veins and veinules (see below). These constrictions of the veinules in the heart must necessarily impede their emptying which occurs during systole, and the blood must be dammed back behind the stenosis. When this occurs near the surfaces of the heart, where the support of the veinules and capillaries is least, ruptures of the wall and consequent hemorrhages take place when the heart contracts. It is possible that a direct injury of the capillary endothelium also occurs in the anaphylactic reaction, such as Heubner (64) postulates for the explanation of capillary hemorrhages after the intravenous injection of widely different chemical substances (salts of the heavy metals, tartar emetic, emetin).

Hemorrhages are not the only gross anatomical changes which are detectable in the anaphylactic heart, though they form the only one described so far for both the guinea-pig and the dog. In the rabbit which has succumbed acutely the right ventricle often shows a gray color, decreased translucency, and a peculiar stiffness of the wall becomes apparent when the right ventricle is slit open for further examination. The right ventricular wall feels firmer than normal on pressure, and this increased firmness is strikingly shown by the resistance of the endocardial surface to the finger nail. If the endocardial surface of the right ventricular wall (not the septal surface) is scraped, the muscle tissue, especially the muscle trabecule of the upper third of the ventricle, resists the finger nail much as if it were connective tissue. The papillary muscles of the right ventricle show a similar resistance, though not as great as that of the wall. The left ventricle, however, shows no indication of this change, and the finger nail easily scrapes off muscle tissue. Similar changes of the cardiac muscle may be produced by intravenous injections of lethal doses of digitalis preparations. Auer (6) interprets these alterations as an intravital rigor.

FUNCTIONAL CHANGES.—The anatomical changes briefly described in the preceding paragraph would naturally lead one to expect some functional alterations as the result of these gross anatomical changes, and such functional alterations are easily detectable.

If the heart of an anaphylactic guinea-pig is examined immediately after respiration has ceased, it will be found contracting vigorously, but the ventricles beat slowly and do not respond to each auricular systole; in other words, there is a state of partial auriculoventricular dissociation.
or block, and the ventricles respond only to every second, third, or even fourth auricular contraction. The finer degrees of dissociation where a ventricular beat drops out after a varying number of complete cardiac cycles obviously cannot be detected by mere inspection. A block, detectable on inspection, was first described by Auer and Lewis (10); it may occur within thirty seconds after the lung has been completely immobilized by the foreign protein, as shown in Figure 1 accompanying this article. The same figure also shows another abrupt change in the cardiac rate occurring about one-half minute after the first one. The strength of the cardiac contraction does not seem much affected, for the ventricles are able to propel the blood many inches into the air when the aorta is cut immediately after the heart has shown some changes in rhythm. According to Auer and Lewis, the block is due to an asphyxia which acts directly on the heart itself, for these alterations in rhythm are just as easily obtained in a pithed animal as in a normal one. While this interpretation is in accord with the action of asphyxia in decapitated atropinized cats (Sherrington, Lewis and Mathison), nevertheless it seems possible that asphyxia is not the only cause of this cardiac block in the guinea-pig because, in the dog and rabbit, block occurs under conditions where systemic asphyxia does not exist.

How early auriculoventricular dissociations occur in the anaphylactic intoxication of the guinea-pig is not known. Unfortunately no electrocardiographic studies have been made so far on this animal. Such experiments offer no technical difficulty, but curarization would be absolutely necessary in order to prevent the convulsions which would mar the proper registration of the heart beats. Studies of this type would also decide whether disturbances of cardiac rhythm or contraction occurred in the non-fatal anaphylactic reaction of guinea-pigs, a question about which nothing is known.

In the rabbit cardiac disturbances play a prominent rôle, and indeed it will be shown that cardiac failure is the cause of death in the acutely fatal cases. When the heart is examined in situ immediately after respiration has ceased, which usually occurs two to five minutes after reinjection in well-sensitized animals, this organ will be found in diastole, the ventricles contracting feebly or not at all, while the auricles beat fairly strongly and at a more rapid rate than the ventricles. Mechanical or faradic stimulation of the ventricles has little or no effect. This loss of contractility of the heart occurs just as swiftly when the rabbit is tested under artificial respiration, when the vagi are cut and after the entire central nervous system has been destroyed. (Auer, 6.) In some experiments the heart may cease to beat abruptly at a time when the blood pressure is excellent and when the curve shows no abnormalities except that the respiratory waves are absent, even though artificial respiration has been maintained throughout. For a tracing of this type see Auer (6), plate 46.
While these functional disturbances, together with the anatomical changes, show clearly that failure of the heart itself causes death in the acutely fatal cases in rabbits, the cardiac changes leading up to the fatal issue had not been investigated with care. For this purpose the electrocardiograph is essential because it permits a careful study of every heart beat from the beginning to the end of an experiment. In an investigation of the anaphylactic rabbit, by means of the electrocardiograph, carried out by Auer and Robinson (11), a variety of alterations in the character and sequence of the heart beat was observed. These authors describe abnormalities which occurred in a great majority of their experiments (22 out of 24). The changes noted, irrespective of whether the vagi were cut or not or whether the issue was death, were (1) alterations in the $P$ wave, which disappeared at times or appeared very close to the $R$ wave, so that the ventricular cycle, the R-T complex, could not possibly be due to the auricular ($P$ wave) impulse; (2) abnormal $R$ waves, the downstroke being slow; (3) the development of prominent $S$ waves; (4) changes in the $T$ wave which disappeared, became negative, or increased in size. These changes in auricular and ventricular activities often occurred without any alteration in the conduction time between auricles and ventricles.

Changes in the conduction time between auricle and ventricle ($P-R$ interval) were observed and led to partial, and even complete, dissociation. This block was only obtained when rabbits with intact vagi succumbed acutely. The dissociations were especially interesting because of their appearance and disappearance, which took place two and even three times, the periods between the dissociation showing a normal sequential beat, though the conduction time was prolonged. Moreover, the electrocardiograms obtained for a short time early in the experiment occasionally showed alterations which seemed identical with those obtained when respiration had ceased, and these changes were of a type which Robinson describes as characteristic of a dying heart: the $T$ waves are sharp, prominent, and occur close to the $R$ waves; the $R$ waves themselves are rather broad, due to a slow downstroke which does not fully reach the base line.

Another interesting alteration which the same authors observed was an abnormal relation between the $P$ and $R$ waves. In seven experiments the conduction time between auricle and ventricle ($P-R$ interval) was temporarily shortened. For example, in an experiment the normal $P-R$ interval was 0.08 second, while two minutes after the injection it had diminished to 0.033 second. This shortening of the interval, like the block, was of temporary duration and, again like the block, sometimes appeared, then disappeared, and again reappeared. Similar changes have been obtained by Rothberger and Winterberg (48) after stimulation of the left accelerator nerve in the dog. Rothberger and Winterberg believe that the power of stimulus formation of the junctional tissue has been raised by stimula-
tion of the accelerator nerve, so that this region becomes the cardiac pace
maker. The same change probably also occurs in the anaphylactic heart,
and the point of origin of the heart beat shifts repeatedly from the sinus
region to the junctional tissue between auricles and ventricles, which ex-
plains the shortening of the P-R interval and the fact that the auricles and
ventricles contract almost simultaneously. It is possible that accelerator
stimulation also plays a rôle in these changes of the anaphylactic heart, for
the cardiac rate usually shows an outspoken augmentation in rate. Never-
theless, the approximation of P and R waves has been observed without
any acceleration. (See Auer and Robinson, plate 35.) This abnormal rela-
tionship between the P and R waves occurred in rabbits with vagi intact
or sectioned, and in fatal as well as in non-fatal cases.

The time of onset of the cardiac changes varied in the different series of
rabbits and occurred soonest in the acutely fatal cases where alterations
were often observable before the injection, which usually lasted about one
minute, was finished. This was especially true of the animals with intact
vagi, while those with sectioned vagi responded within three-quarters to
two and a half minutes after the beginning of the injection. No such
difference was, however, noted in the non-fatal cases; there the alterations
appeared within one to five minutes after the beginning of the injection,
irrespective of whether the vagi were cut or intact. No definite statement
can therefore be made regarding the influence of the vagi on the onset of
the cardiac symptoms.

The cardiac changes recorded by the electrocardiograph occurred in the
fatal cases before respiration ceased, and therefore cannot be attributed to
an asphyxia. This inference is still further strengthened by the non-fatal
experiments where the respiration was never embarrassed, although the
electrocardiograms showed a variety of abnormalities.

The duration of these changes varied; in the fatal cases they appeared,
lasted a short time, and disappeared, to appear again after a period of
normal beats. This continued until the animal died. In the non-fatal
cases with vagi intact the abnormalities lasted 7 to 21 minutes; in the
series with vagi cut the duration was shorter, only 2½ to 5 minutes. This
difference seems to indicate that some effect is exerted upon the vagus
center during the anaphylactic reaction.

That the electrocardiographic abnormalities were really of anaphylac-
tic origin Auer and Robinson demonstrated by failing to obtain them when
the antigen (horse serum in this instance) was reinjected intravenously
into sensitized animals after the effects of the first reinjection had passed
off and when the animals were therefore antianaphylactic. Normal rab-
bits also failed to show the characteristic changes when injected with horse
serum, but in one of these controls premature, ectopic beats developed. As
these extrasystoles were also observed in a sensitized rabbit which had
been again reinjected immediately after recovery from the first intoxica-
ing dose, Auer and Robinson are inclined to regard these extrasystoles as probably not significant when they occur in the anaphylactic state.

Hecht and Wengraf (62) have recently examined young rabbits with the electrocardiograph during horse serum anaphylaxis. The main disturbance these authors observed were extrasystoles of the apical type; they also noted negative P waves, flattened or negative T waves, and the development of S waves. Disturbances of conduction or the development of block were not obtained by them.

Alterations in the rate of the heart beat appear most sharply, like most anaphylactic reactions, when the reinjection is given intravenously. If the blood pressure of an anaphylactic rabbit is recorded by means of a membrane manometer, which gives a fairly accurate picture of the individual pressure pulse beats, the following alterations may be observed. Toward the end, or shortly after the reinjection, the heart slows moderately; this slowing lasts less than a minute and suddenly gives way to a very rapid small pulse. This rapid pulse may persist with a gradually sinking blood pressure until the heart stops beating. As a rule, however, the rapid pulse rate is interrupted by short stretches of large, slow pulses. As the initial slowing of the rate is obtained just as well in rabbits with vagi cut as in those with vagi intact, the effect cannot be of central origin, but must be peripheral, and occurs perhaps in the vagus endings of the heart itself. The increase in rate which occurs later may possibly be due to a stimulation of the accelerator nerves; whether this stimulation is peripheral, acting on the accelerator cardiac endings, or whether the effect is exerted centrally in the medulla, cannot be decided with the evidence.
available at present. It has already been stated that this acceleration may have some relation to the approximation of P and R waves, which was noticed first by Rothberger and Winterberg.

The dog also shows cardiac derangements which are directly attributable to the anaphylactic reaction. That the heart is involved is already indicated by the fact that this organ shows a definite abnormal reduction in direct irritability when examined immediately after acute anaphylactic death. Moreover, the location of subendocardial hemorrhages in the conducting system, which have already been described, would also lead one to expect some functional expression for these anatomical changes.

FIG. 4.—Dissociation. Onset of Partial Auriculo-ventricular Dissociation. Three minutes after injection of 20 c.c. horse serum into the external jugular vein. Auricular rate, 267. One auricular beat in every eight is blocked. Conduction time varies from 0.12 to 0.28 second. Note diminution of R wave and increase of S wave.

FIG. 5.—Partial Auriculo-ventricular Dissociation of a Higher Degree. Nine minutes after injection of the horse serum. One auricular beat in every four is blocked; Auricular rate, 233. Conduction varies from 0.14 to 0.26 second.

ordinary methods, however, failed to detect any primary anaphylactic effect on the heart of dogs. Biedl and Kraus (22) never observed any cardiac irregularity at any time during the reaction, and they emphasize
the fact that a slowed and perhaps irregular activity of the heart is replaced by a remarkable regularity during the stage of low blood pressure. Eisenbrey and Pearce (44) tested the question experimentally and recorded the heart’s activity by means of a Cushny myocardiograph. They found no evidence that the functional activity of the sensitized dog’s heart was primarily affected by the injection of the toxic dose. Certain changes which occurred in the myocardiograph tracing after a low blood pressure level had been reached Eisenbrey and Pearce observed to be due to an incomplete filling of the right heart; both the right auricle and right ventricle showed a marked decrease in size, and the right ventricular wall appeared flabby and collapsed during diastole, but contracted in rate, extent, and regularity just as it did before the injection.

![Graph](image)

**Figure 6.**—**Partial Auriculo-Ventricular Dissociation due to Anaphylaxis.** Twenty-nine minutes after injection. The normal sequential beat has returned, but the form is still abnormal. Conduction time, 0.12 second. Rate, 240.

Positive evidence that the heart of the anaphylactic dog may show irregularities was brought forward by Robinson and Auer (105). They examined the animals by means of the Edelmann large model electrocardiograph, and the electrodes were applied to the right front and the left hind leg (lead 2). These authors found that cardiac disturbances are much less frequent in the dog than in the rabbit, where the anaphylactic reaction almost invariably brought on some cardiac change. Out of twelve dogs only six exhibited well-marked pathological electrocardiograms, and these occurred whether the vagi were intact or sectioned at the time the intravenous reinjection was given. All of these animals showed disturbances of the conduction time (P-R interval). In five the P-R interval was lengthened, and in two animals this lengthening was so marked that partial auriculoventricular dissociation of varying degree took place. Figures 3 to 6 illustrate two stages of partial heart block obtained from one dog. In Figure 4 every eighth auricular impulse is blocked, and in Figure 5, a later stage, every fourth auricular impulse fails to produce a ventricular
contraction. The conduction time varied between 0.12 and 0.28 second during the block, while normally it was 0.08 second.

In one animal with intact vagi the P-R interval was practically abolished and auricles and ventricles beat synchronously. This occurred with a blood pressure of 30 mm. of mercury while the heart was beating 148 per minute. The P and R waves gradually separated, the P waves being negative at one stage, and thirteen minutes after the onset the electrocardiogram was normal, the P-R time was 0.10 second, the rate 167, and the blood pressure 40 mm. This type of alteration has already been discussed in the paragraphs dealing with cardiac disturbances in the anaphylactic rabbit, where it occurs more frequently, and attention was there called to the similar changes which Rothberger and Winterberg (46) obtained when the left accelerator nerve was stimulated in dogs. In the anaphylactic dog, however, acceleration of the heart rate has been but rarely observed during the anaphylactic reaction, and in the case cited above the heart was strongly slowed, from 210, the normal rate, to 184 per minute at the time when auricles and ventricles beat synchronously.

In addition to changes in the P-R interval the form of the electrocardiograms was altered. Four experiments showed well-defined abnormal ventricular complexes of the same general type. The changes consisted of a diminution of the R waves, a marked deepening and splitting of the S waves, and an exaggeration of the T waves, which sometimes partly fused with the S waves. This change of form, illustrated in Figures 5 and 6, appeared gradually during the anaphylactic reaction, reached a maximum, and then usually returned to the form obtained before the reinjection of the foreign protein. As these changes resemble closely those which Eppinger and Rothberger (45) obtained in the dog when a 20 per cent. solution of silver nitrate solution was injected directly into the wall of the right ventricle, or when the limb of the His bundle leading to the right ventricle was cut, it seems legitimate to assume that some alteration occurs in the musculature of the heart during the anaphylactic reaction. This alteration may be caused by the hemorrhages which have been shown to occur into the conducting system during the anaphylactic reaction.

That these deviations from the normal type of the electrocardiogram observed in the dog were true anaphylactic changes was demonstrated by their non-appearance when the animals were again reinjected after the effects of the first reinjection had largely disappeared. Such an injection in the antianaphylactic state produced no effect upon the form of the electrocardiogram, nor upon the blood pressure. Nor did the same amount of the same foreign serum, when injected into normal dogs, cause changes in the electrocardiogram which, even remotely, resembled those observed during the anaphylactic reaction.

It might be thought the profound drop in blood pressure which appears in the anaphylactic dog was the primary cause of the electrocardiographic
alterations described above, because a more or less pronounced anemia of the cardiac muscle might ensue as a result of this lowered blood-pressure level. The experiments showed, however, no relationship between the drop of blood pressure and the appearance and severity of the electrocardiographic alterations. Some of the anaphylactic dogs which exhibited remarkable drops in blood pressure (145 mm. Hg within 45 seconds in one instance) nevertheless exhibited no change in the form of the electrocardiogram, and the changes in the conduction time (P-R interval), when present, sometimes occurred early, sometimes late, during the state of low blood pressure. These facts, together with the observations that sudden lowerings of the blood pressure level by means of amyl nitrite, sodium nitrite, with or without section of the splanchnic nerves, produced no changes in the electrocardiogram which were at all comparable to those obtained during the anaphylactic state, led Robinson and Auer to conclude that the blood-pressure changes themselves did not cause the electrocardiographic changes, but that these changes were of a primary anaphylactic nature.

The alterations observable in the electrocardiogram develop more or less gradually; they usually begin within a few minutes, or even seconds, after the injection; the maximum is usually reached within fifteen minutes, and after the lapse of thirty minutes the electrocardiogram is practically normal. Occasionally the entire process occurs more speedily, and the period of abnormal cardiac activity appears in less than one minute after the injection, persists for a few minutes, and then disappears practically within five minutes, although the animal may succumb. The changes in the heart of the dog are therefore reversible as in the rabbit, but the dog does not apparently show the repeated oscillations between normal and abnormal complexes, such as occur in rabbits, although rhythmic oscillations in the size of the P and T waves do take place.

**Rate.**—The statements in the literature vary concerning the cardiac rate during the anaphylactic reaction of the dog. Biedl and Kraus (22) report a well-marked increase in the cardiac rate, beginning with the drop in blood pressure; the tables of Arthus (3) show but slight changes, while Robinson and Auer (105) saw a more or less marked decrease in the majority of their experiments. The differences may perhaps be due to differences in technique, especially anesthesia.

In the cat Schultz (115) observed that cardiac irregularities appeared shortly after the intravenous injection of horse serum. The right auricle, right ventricle, and pulmonary artery become gorged with blood, while the left side of the heart is practically empty. By massaging the heart and forcing blood through the pulmonary artery several animals survived, according to Schultz.

In frogs sensitized by the injection 0.1 to 0.5 sheep serum into a vein, or into the dorsal lymph sac, and reinjected intravenously after 1 to 4 weeks, Friedberger and Mita (50) observed that the animals became weak.
THE FUNCTIONAL ANALYSIS OF ANAPHYLAXIS

and were unable to hop. Acute death never occurred, but the majority of the animals died within 12 to 24 hours. If the chest was opened so that the heart could be inspected and its action recorded graphically, the heart showed a gradually developing strong slowing in the rate of beat due to increased length of diastole, and a marked diminution of the amplitude of contraction. Irregularities of the heart beat were also observed. Normal frogs did not react when the same quantity of sheep serum was injected intravenously.

Experiments upon the Isolated Heart.—Cesaris Demel and Launoy report the effect of perfusing the isolated hearts of sensitized rabbits and guinea-pigs with the protein used for sensitizing. The results of Launoy are especially convincing. This author perfused the coronary vessels of the excised heart of sensitized guinea-pigs with 20 per cent. horse serum in Ringer-Locke solution. The anaphylactic reaction could be obtained in 90 per cent. of the cases, and showed the following characteristics: After the diluted serum reaches the heart the organ contracts more swiftly and the amplitude increases. This phase, which may be only slightly marked or entirely absent, lasts a short time, and is succeeded by an abrupt slowing with or without increase in the amplitude. Now follows an increasing diminution of the amplitude of contractions, together with an increase of the diastolic pauses, which may lead to a stoppage in diastole; the myocardium remains irritable. In most experiments, however, stoppage does not occur, but the heart soon after the initial disturbances beats like a normal organ, although the circulation of the serum solution is continued. This anaphylaxis can also be demonstrated when the heart of a guinea-pig is perfused shortly after the animal has recovered from the anaphylactic reaction; here also the heart continues to beat regularly and strongly when serum is added to the perfusion liquid, and there is no evidence of any disturbance whatsoever. Cesaris Demel’s results in the rabbit differ from those of Launoy chiefly in the fact that the Italian observer noted effects in the sensitized heart which were merely more pronounced than similar effects observable in the normal heart after perfusion with horse serum. Launoy, on the other hand, emphasizes the fact that horse serum exerts a depressing action on the sensitized guinea-pig’s heart, but a tonic action in the normal heart.

Extracardiac Circulatory System.—Blood Pressure.—Changes in the blood pressure during the anaphylactic reaction were first noticed by Richet (98) in 1902. He observed that the intravenous injection of a certain amount of actinotoxin solution did not alter the blood pressure of a normal dog; but the same dose injected intravenously into a dog who had been treated three or four weeks before with the same actinotoxin now caused a drop in blood pressure. The drop in pressure developed within 2 to 3 minutes after the injection and amounted to 80-100 mm. of mercury. As the change in blood pressure occurred only after some minutes, and as
atropin did not prevent it, Richet believed that the heart itself was not affected.

The first investigators, however, to demonstrate that a drop in blood pressure is one of the most constant phenomena in serum anaphylaxis of the dog and rabbit were Biedl and Kraus (19) and Arthus, and their objective findings have been corroborated almost entirely by later investigators.

In dogs the changes were carefully analyzed, especially by Biedl and Kraus. They found that dogs sensitized by the subcutaneous injection of

![Diagram](https://via.placeholder.com/150)

**Fig. 7.—Anaphylactic Drop of Blood Pressure in Dog.** Dog 9 6500; sensitized with 10 c.c. horse serum injected subcutaneously, 5 c.c. in each flank. After 30 days the animal was etherized fully and the blood pressure recorded by a mercury manometer from the carotid artery; half-saturated sodium sulphate solution filled the connecting tubing. Time recorded in 6-second intervals. Time line, O pressure line.

The reinjection of horse serum is marked by the broad white band above the time line; 20 c.c. horse serum were injected into a jugular vein.

The blood pressure falls abruptly from 126 mm. to 10 mm. within one minute after the beginning of the serum injection, and spontaneous respiration ceased. The dog succumbed swiftly although intratracheal insufflation was maintained. On immediate autopsy the heart was motionless in diastole and did not respond to mechanical stimuli. The lungs resembled the typical asthmatic lung found in the anaphylactic guinea-pig. (See Fig. 2.)

horse or bovine serum and reinjected intravenously after three weeks showed within fifteen to thirty seconds a gradually increasing lowering of the blood pressure, accompanied by a general excitation of the animal. The pressure may sink from a normal level of 120-150 mm. of mercury (femoral artery) to 40 mm. and less. At this low level the oscillations of the curve due to respiration may be strongly decreased or entirely absent, and the individual pulse beats are much smaller and more rapid than normal. The period of low pressure coincides with the stage of general depression of the dog. If the animal survives, the blood pressure gradually rises and reaches its normal level within one or more hours. Biedl and
Kraus noted a marked parallelism between the degree of blood-pressure depression and the clinical picture: the lower the pressure sinks the severer the picture of intoxication.

Similar observations in sensitized dogs were made by Arthus, who observed that the drop occurred in pronounced cases within 50 to 85 seconds after the injection. This drop reached a low level within 50 to 85 seconds, and remained stationary for a variable period, at times only a few minutes. Arthus observed repeatedly that the original level was re-attained 10-25 minutes after the injection; this result is probably to be ascribed to the relatively low sensitization of Arthus' animals.

In the dog there is no marked difference except one of degree in the blood pressure curve obtained from those which survive and those which succumb.

In rabbits sensitized subcutaneously with horse serum and reinjected intravenously, and which survived an intravenous reinjection, Arthus observed, as a rule, a very similar blood pressure picture: 15-25 seconds after the injection the carotid blood pressure falls from the normal level of 100-120 mm. of mercury to the 34 to 48 mm. level. This level is reached within 15-45 seconds after the pressure begins to fall, and is maintained for about 20-25 minutes. Arthus also observed a marked diminution of the respiratory and cardiac oscillations during the drop in pressure, so that the curve almost appeared as an unbroken line (mercury manometer).

Arthus does not mention the occurrence of any rise of blood pressure in the rabbit immediately after the injection. Such a rise, however, was noted fairly frequently by Loewit (79) and Auer (9). This rise was moderately slow, rarely exceeded 20 mm. of mercury, persisted after the injection, and could not be attributed to the mechanical effect of the injection itself.

If a rabbit succumbs acutely to the reinjection the blood-pressure curve is somewhat different from that just described. Shortly after the reinjection of horse serum the blood pressure often begins to rise, the pulse pressure increases, the respiratory oscillations become less or disappear, and the heart slows moderately. This rise, which may be 20 mm. and more, does not last longer than one minute, and is often broken by a series of drops which look like vagus pulses, though they are also obtained in animals whose vagi have been sectioned. Then the pressure slowly sinks, the pulse pressure decreasing strongly, while the rate usually increases. This drop may continue until within one to two minutes the 10 to 15 mm. level is reached, and after 5 to 6 minutes no heart beats are discernible on the curve, even though the record be taken with a membrane manometer. During the final drop in this type of curve the record always shows arrhythmias and marked sudden changes in rate and in pulse pressure. A modification of this type is introduced when the abrupt increase in the
pulse rate, which occurs after the initial slowing, temporarily delays and slows the drop in blood pressure, but here also the membrane manometer records no beats within five to ten minutes after the reinjection. Still another modification of the curve is obtained when the heart abruptly stops beating, which occurs now and then. All these forms of blood-pressure curve are obtainable from animals which have been curarized, and whose vagi have been cut in the neck previous to the reinjection. (Auer, 6.)

In the cat the blood-pressure curve is quite similar to that obtained in the dog, according to Schultz (115), but the reaction is apparently more severe, for his curves show practically no pulse beats, even before the lowest level is reached.

In the guinea-pig which dies acutely the blood pressure rises gradually during one or two minutes after the injection. This rise varies from 20 to 60 mm. of mercury, and is usually associated with an increased pulse pressure and slight alterations in rate; then a gradual drop in the blood pressure follows, usually with a marked slowing of the heart, and the 10 mm. level is usually reached within five to ten minutes after the reinjection. The pulse pressure decreases during the drop, and at the lowest level the individual heart beats can hardly be distinguished, even when recorded with a membrane manometer. (Auer and Lewis; Biedl and Kraus, 22; Loewit, 79.)

The course of the blood-pressure curve in a non-fatal reaction of the guinea-pig has not been described as far as the writer is aware.

From the preceding descriptions two general types of blood-pressure reaction can be distinguished: (1) the abrupt deep fall of blood pressure which occurs within one minute after the injection and reaches its maximum within another minute or two, such as occurs in dogs and cats; and (2) the slower, more protracted lowering of the blood pressure usually preceded by a rise such as occurs in the fatal reaction of rabbits and guinea-pigs. To this group the writer would also add on the basis of his experiments the blood-pressure reaction of non-fatal anaphylaxis in the rabbit, although Arthus' description indicates a close likeness to the type which occurs in the dog. These different types of blood-pressure reaction are apparently caused by different mechanisms.

Biedl and Kraus (19) came to the conclusion that the blood-pressure drop in the dog was caused by a transitory paralysis of the peripheral vasomotor apparatus in the splanchnic area. They excluded the heart as a possible factor on theoretical grounds, but were substantially correct in this, for the direct registration of ventricular activity by Eisenbrey and Pearce (44) showed no decrease in rate and strength during the early stages, and the electrocardiographic studies of Robinson and Auer revealed no definite relation between a pathological activity of the heart and the abrupt decrease in arterial pressure; moreover, a number of their dogs exhibited a profound blood-pressure effect without any altera-
tion of the electrocardiogram. It is legitimate, therefore, to exclude the heart as a vital factor in the production of the blood-pressure drop. Biedl and Kraus' experimental proof was as follows: during the stage of low blood pressure in the dog stimulation of the peripheral stumps of the splanchnic nerves gave no rise in blood pressure; the intravenous injection of 1 to 2 c.c. of adrenalin had only a slight or no effect in the early stage of arterial depression, though a gradually increasing rise of pressure was obtained as the dog recovered; the injection of BaCl₂, however, raised the blood pressure, even when injected very early in the stage of arterial depression. Since adrenalin is believed to act chiefly upon the vasomotor endings, while BaCl₂ acts by stimulation of the vascular musculature itself, Biedl and Kraus' inference was well founded, and has been corroborated and amplified by other investigators, especially Pearce and Eisenbrey (92). Pearce and Eisenbrey also demonstrated that, with the decrease in arterial pressure, the kidney, intestine, and spleen show a decrease in volume, while the blood accumulates in the large venous trunks and in the liver. The accumulation of blood in the liver was graphically registered by Edmunds (42). Pearce and Eisenbrey characterize the condition of anaphylactic low blood pressure in the dog as a bleeding into the veins of the abdomen, analogous in many respects to surgical shock.

For the cat Schultz (115) states that the drop in blood pressure is caused by a weakening of the heart, especially the right side, which becomes distended with blood and loses its power of contraction almost immediately after the horse serum is injected intravenously, together with a constriction in the divisions of the pulmonary artery so that little blood enters the left auricle. Schultz explains the venous congestion of the splanchnic area as due to back pressure, because the right side of the heart is unable to empty itself on account of its weakness and the increased resistance in the pulmonary arterial circuit. Similar results were obtained by Schultz after clamping arteries and veins so that the circulation was practically limited to the heart-lung circuit. The evidence undoubtedly shows that the heart is strongly affected in the cat, but it does not prove that the splanchnic congestion is purely a passive effect. Moreover, it must be emphasized that Schultz does not discriminate sharply between the effects observed on first injection of horse serum in cats and those which occur when sensitized animals are reinjected; he apparently considers the primarily toxic action of horse serum in cats as qualitatively identical with the action which the serum produces when injected into cats sensitized with this serum.

This back pressure theory of Schultz does not hold for the dog, for Pearce and Eisenbrey saw no distention, but a collapse, of the right side of the heart during the blood-pressure drop, and Edmunds (42) at that time observed only a transitory rise of pressure in the pul-
monary artery and pulmonary veins, followed immediately by a drop, indicating no stenosis in the pulmonary circuit.

In the acutely fatal anaphylactic reaction of the rabbit the heart plays an undoubted rôle in the causation of the drop of blood pressure, for the gross muscular changes which strongly reduce, and even abolish, cardiac contractility must obviously have this effect. What rôle the splanchnic motor endings play in this animal has not been established with certainty; but Scott (121) observed that an intravenous injection of adrenalin during the stage of low pressure produces only a transitory rise of pressure without amelioration of the symptoms. That some effect is exerted upon the splanchnic area is also indicated by the often intense engorgement of the liver and of the portal system of vessels. Perhaps the anaphylactic intoxication in the rabbit does not act equally upon the heart and the splanchnic area, and the different degrees with which they respond may explain the different types of blood-pressure drop which have been described for this animal. The initial rise of blood pressure may possibly be due to a stimulation of the vasomotor center, as Loewit (79) suggests, but this is not established with certainty.

In the guinea-pig the blood-pressure changes are probably secondary to the asphyxia which develops within a few seconds after the reinjection. The heart, although it often shows extensive hemorrhages, shows no weakness, but almost invariably beats powerfully on inspection when the blood pressure is not more than 10 to 20 mm. of mercury, and drives blood some inches into the air when the pulmonary artery or the aorta is cut open. The splanchnic area often shows marked engorgement, but this is by no means invariable; in the same series of animals one may observe the small intestines quite pale and contracted and the mesenteric vessels practically empty, while others show a pronounced congestion, especially of the mesenteric vessels.

In general it may be said that in the guinea-pig, as well as the rabbit, the rôle of the splanchnic area as a factor in the blood pressure has not been sufficiently studied, and the warning of Biedl and Kraus not to identify indiscriminately the lowering of the blood pressure during anaphylaxis in the dog, rabbit, and guinea-pig is still justified.

Other Changes in the Circulatory Apparatus.—Schultz and Jordan (119) observed that the arterioles in the anaphylactic lung of the guinea-pig show a series of constrictions so that the artery looks beaded, and the lumen is practically obliterated. This condition was apparently observed only in the guinea-pig, and was noted in normal as well as anaphylactic lungs. Similar observations have recently been described by Fröhlich (54) in the mesenteric arterioles and small veins of frogs. The frogs had been sensitized by the injection of 0.1 to 0.5 c. c. of pig or sheep serum into the dorsal lymph sac, and the test was made 8 to 15 days later by applying a dried flake of the homologous serum locally on the exposed
mesentery of the curarized animal. Microscopical examination showed gradually developing contraction rings of the arteries and veins. Fröhlich also observed changes in the capillaries in the neighborhood of the serum; after 10 to 15 seconds they became maximally dilated and irregularly contoured; some of the capillaries were full of red corpuscles, while others were filled with clear plasma. Beading of the veins may also be observed quite frequently in the small veins of the gut, mesentery, and diaphragm of guinea-pigs and rabbits who succumb acutely to the anaphylactic reaction; it is usually especially obvious in the large veins which border the central tendon of the diaphragm (Auer, 9). It is probable that these beadings play a rôle in the production of the superficial hemorrhages of the heart, spleen, lung, and gastro-intestinal canal described by Gay and Southard.

A marked dilatation of the conjunctival vessels has been described by Denecke in dogs sensitized and intoxicated by the intravenous injection of egg white. Within 5 to 7 minutes after the reinjection the conjunctival vessels dilate strongly, and the dilatation may persist for half an hour.

**Muscle System.**—Smooth Muscle of the Viscera.—The smooth muscle of the guinea-pig's lungs, or the musculature of the arteries and veins, are not the only places where an anaphylactic reaction occurs in smooth muscle. Schultz (115, 116, 117), in an important series of investigations, was the first to show that smooth muscle in general from the intestine, bladder, and arteries exhibits an anaphylactic reaction, but unfortunately he did not differentiate clearly between a true anaphylactic reaction obtainable only in a sensitized animal and the similar reaction which native sera sometimes exert. As Schultz's work was corroborated, corrected, and amplified later by Dale (33, 34), and as Dale deals only with true anaphylactic phenomena, the following description is based on Dale's work.

Dale employed the horns of the uterus from virgin guinea-pigs sensitized with various proteins, chiefly horse serum, because he found this organ responded more regularly and delicately than any other smooth muscle preparation from the guinea-pig. After suspension in warm oxygenated Ringer solution, and connection with a writing lever, the horn soon loses tonus and exhibits a small fairly rhythmic series of contractions. The irritability of the preparation remains practically unimpaired for some hours. If to such a preparation the protein used for sensitization is added, the uterus responds with a strong tetanic contraction, which is maintained a varying length of time, and is followed by a slow relaxation. The doses necessary to obtain specific responses were very small; curves illustrate the article which show a strong contraction when 0.0001 c. c. of horse serum was added to the bath volume of 50 c. c. Ringer solution, which represents a dilution of 1-500,000. Even greater dilutions, for example, 1-1,000,000 of horse serum, produced a definite, though not maximal, response. Dale states that, as a rule, the uteri of animals sen-
sitized by small doses of horse serum and tested after twelve days show a strong response to dilution of horse serum above 1-100,000.

After the sensitized uterus preparation has responded maximally to the protein used for sensitization it does not contract again, after relaxation and change of bath solution, when the same protein is added in even stronger concentration; it is desensitized or anitnaphylactic. A non-specific contraction may, however, be obtained by the addition of sera containing toxic constituents (fresh horse or guinea-pig serum), and such contractions are also obtained when the same sera are allowed to act upon normal non-sensitized uteri.

Dale was also able to resensitize his preparation after it had become specifically refractory or anitnaphylactic. This was accomplished by allowing the uterus to remain for several hours in an oxygenated 10 per cent. solution of fresh serum from a guinea-pig sensitized with horse serum. After thorough washing with Ringer solution this preparation gave a definite response when subjected to the action of a 1-400 solution of horse serum. A further test showed that desensitization or anitnaphylaxis had now again been established. Passive sensitization of the normal uterus was, however, only obtained when the organ was perfused through its arterial system for several hours with a 20 per cent. solution of serum obtained from guinea-pigs sensitized to horse serum. On testing, the uterus horn responded typically to a horse serum dilatation of 1-500 Ringer, while the control horn, which had not been perfused, showed no effect whatsoever.

The uterine preparation, therefore, permits the demonstration of many of the fundamental phenomena of anaphylaxis; passive sensitization, specific reaction, antianaphylaxis, and even the period of incubation is indicated.

During the anaphylactic reaction there are a number of other phenomena which are referable to a tetanic contraction of smooth muscle. All observers have noted the roughening of the fur in anaphylactic guinea-pigs, and a similar effect may be observed in rabbits. This erection of the hair may be due to an anaphylactic contraction of the pilomotor muscles, though no rigid proof has yet been given.

The scrotum of sensitized dogs when reinjected often shows a slow, powerful contraction which produced marked corrugations of the scrotal sac. (Auer, 9.)

The iris may show a strong constriction during the anaphylactic intoxication. Schultz (115) observed that the pupils of a normal non-sensitized cat diminished to a slit after horse serum had been injected intravenously. A similar strong effect may be observed in rabbits sensitized to horse serum. When the antigen is reinjected the pupils often become pinpoint in size.

The tetanus produced in smooth muscle by the anaphylactic reaction
seems to last about the same length of time, no matter what the origin of the muscles. Dale's experiments with the uterine horns of guinea-pigs show that approximately 5 to 20 minutes elapsed before the structure was normally relaxed again. A similar interval is to be noted in Schultz's work with intestinal smooth muscle. The scrotal sac assumes its smooth surface approximately 5 minutes after the contraction has begun. The contraction of the iris lasts from 5 to 15 minutes when the innervation is intact, and about 30 minutes when the dilator pupils is denervated by extirpation of the superior cervical ganglion. The time interval for the bronchial muscle cannot be judged accurately, but the anaphylactic lung of the guinea-pig largely maintains its distention for days when kept in the ice-chest; at room temperature a definite diminution in size is observable within one hour as a rule (Auer, 9).

Striated Muscle.—A number of functional and anatomical changes in the heart and striated muscles of anaphylactic animals, chiefly guinea-pigs and rabbits, have been described by Gay and Southard (55), Auer (8), Beneke and Steinschneider (14), Loewit (80), and v. Worzikowsky-Kundratitz (144). Gay and Southard (55) in 1907 observed fatty changes and hemorrhages in the heart and voluntary muscles of guinea-pigs which succumbed to the reinjection. In addition the voluntary muscles of fore- and hind-legs showed swelling and loss of striation microscopically. Changes in the heart muscle of rabbits which succumb acutely have already been considered; they consist chiefly of a loss of irritability of both ventricles, together with a rigor-like alteration of the right ventricle, which is not found in the left ventricle. Rigor-like changes may also be observed in the diaphragm and thigh muscles of the rabbit. (Auer, 6.) A speedy development of rigor in the guinea-pig's heart has been described by Loewit, though this does not occur abruptly during life as in the rabbit, but only after the heart has gradually stopped beating. The histological examination of guinea-pigs' hearts by v. Worzikowsky-Kundratitz showed findings which were quite similar to those observed by Beneke and Steinschneider in the diaphragm and skeletal muscles of anaphylactic guinea-pigs, though quantitatively less marked. Beneke and Steinschneider describe a granular waxy degeneration of the muscle fibers, while Worzikowsky-Kundratitz saw a waxy degeneration only occasionally, the most constant change in his experience being a cloudy swelling with granular degeneration. The degeneration was most pronounced in the diaphragm, where the majority of the muscle fibers look swollen, show a loss of striation, and present a homogeneous, cloudy, occasionally granular appearance. Beneke and Steinschneider considered these changes the direct result of an anaphylactic poison, but Wells (141) pointed out that this interpretation is improbable because a typical waxy degeneration of striated muscle may be obtained by a lengthy stimulation of its motor nerve, and is attributable to the formation of sarcolactic acid. As ana-
phylactic guinea-pigs die of an asphyxia associated with violent convulsions, conditions are favorable for a maximal accumulation of sarcoclastic acid in the muscles, which Wells has experimentally shown to be capable of producing the histological changes described.

As the histological alterations are much more pronounced in the anaphylactic animals than in those killed by peptone, nucleic acid solution, or primarily toxic sera, v. Worzikowsky-Kundratitz is inclined to consider the intensity of the reaction as characteristic for the anaphylactic intoxication.

Gastro-intestinal System.—The stomach and intestines exhibit obvious anatomical and functional alterations during the anaphylactic reaction, the character of which varies with the animal species employed. In the dog Richet (100, 101), Biedl and Kraus (19, 22), and Pearce and Eisenbrey (92) noted the following effects: The first symptom usually is retching and vomiting, which may begin within a few seconds after the animal has been injected. The severity of this vomiting seems especially great in dogs reinjected with poisonous animal extracts, for Richet describes the vomitus as sometimes fecal and even mixed with blood. A few minutes after the onset of the vomiting, evacuations of the bowel occur which are fluid and sometimes stained with blood. The bladder is also emptied. In this stage the animal is usually lying limply on the floor, the respiration is usually deepened, but, as a rule, no strong dyspnea is present. The animal does not respond to a call, but is not unconscious; it merely exhibits a marked muscular weakness. In the average dog sensitized with horse serum the attacks of vomiting become gradually less severe, and may disappear within fifteen minutes after the injection. The diarrhea, however, may persist for many hours. On autopsy Pearce and Eisenbrey observed swollen and hemorrhagic areas in the mucosa along the greater curvature of the stomach, and a similar condition in the duodenum and upper small intestine; the Peyer’s patches were dark and elevated, but showed no hemorrhages; the colon was also hemorrhagic.

In the rabbit vomiting cannot occur because the stomach content is semi-solid, but the intestine and cecum show marked peristaltic movements which are easily visible through the relaxed abdominal walls of the animal. This increased peristalsis is not limited to the small gut and cecum, but also occurs in the colon, for shortly after the injection dry well-formed scybala are passed. The quantity of feces evacuated varies considerably in different rabbits; a considerable number of pellets may be obtained from one rabbit, while its mate, which was treated in exactly the same way, passes only a few.

Peristalsis is best observed in rabbits which have been stretched out on their backs and the abdominal hair clipped. The normal peristaltic and antiperistaltic waves of the cecum are markedly increased in strength and frequency, and evidences of small-intestinal activity are seen in the
left upper and right lower quadrants of the abdomen. The intestinal activity due to the reinjection usually begins shortly after the intravenous injection during the stage of rapid shallow respiration. Arthus (2), who first observed the increased peristalsis in the rabbit, states that the pellets are absolutely normal, and that there is no diarrhea. Auer has observed the same, but Scott (121) has described the appearance of a thin watery diarrhea. Autopsy does not show any pronounced changes as a rule: there is slight or no peristalsis; the gut may be moderately congested, but the mesenteric vessels, especially the veins, are usually large and full. The surface of the small intestine and cecum may show some hemorrhages. Scott describes a marked capillary engorgement with minute hemorrhages, which are especially noticeable in the intestinal villi.

In the guinea-pig gastro-intestinal symptoms are still less marked than in the rabbit. True vomiting does not occur, but in animals which have been stretched out on their backs for examination stomach contents may often be observed in the mouth during the violent asphyctic convulsions which the reinjection causes. This material has probably been forced out of the stomach by the strong compression which the stomach suffers when the costal margin and sternum are drawn inward during an inspiratory attempt, and the increased negative pressure in the thorax, and consequently esophagus, must also aid in bringing material from the stomach back into the mouth. Fecal pellets begin to appear usually after the first signs of asphyxia develop, but the entire quantity passed is usually small. The pellets are always well formed and no true diarrhea has been recorded. Visible peristalsis occurs after the animal has succumbed and the abdominal walls are relaxed. When the abdomen is opened the small intestines at times contract violently, but coördinately, and a strong wave of contraction which constricts and blanches the gut to a gray cord sweeps swiftly down, driving the fluid contents before it with such speed that the loop of intestine rises up and remains standing for a second or so like a wire spring because the relaxation takes place with some slowness. While this type of intestinal peristalsis (Rollbewegungen of Houtkeest) is surely partly due to asphyxia, it seems probable in view of the work of Schultz and Dale that it is also partly an anaphylactic phenomenon.

The gut itself is usually found moderately congested, but in many instances it may be quite pale and relaxed without any noticeable hemorrhages at all.

Whether or not hemorrhages are pronounced in the gastro-intestinal canal (Gay and Southard) seems to depend to some extent upon the speed with which death results; the more rapid the death the less prominent the hemorrhages often are. After intraperitoneal reinjection the guinea pig usually dies within an hour, and Gay and Southard found that gastric hemorrhages were especially frequent, though not necessarily constant. These gastric hemorrhages, varying in size from a pin point to 2 cm. in
diameter, occur chiefly on the greater curvature, and are submucous or show definite erosion, with hemorrhages into the stomach. The same authors also observed hemorrhages in the cecum, lung, spleen, adrenals, heart, and diaphragm. Histologically Gay and Southard describe minute interstitial hemorrhages due to endothelial fatty changes in the capillaries.

Glandular System.—Anatomical and functional changes have been described in glandular structures. Modrakowski observed increased secretion of pancreatic juice in the dog during the anaphylactic reaction. The secretory activity of the tear and salivary glands is also somewhat augmented.

The adrenal glands of guinea-pigs which have succumbed or recovered from an anaphylactic action show an intense diffuse green coloration after fixation in Müller-formalin, while controls exhibit only a slight green color, according to Uecke. This author tentatively advances the suggestion that the drop in blood pressure is due to a fixation of adrenalin in the glands.

Necrosis of varying types has been described in the kidney and liver by Gay and Southard (55) and others. Longcope has recently again investigated this question in the guinea-pig, rabbit, cat, and dog. All the animals were sensitized by repeated injections, usually subcutaneously, of horse serum or egg-white. The toxic reinjection was administered usually intravenously. Longcope observed especially the kidney, and records practically similar changes in all species of animals examined: marked nephritis with degeneration and necrosis of the loop of Henle, collecting tubules, occasionally also the convoluted tubules. These alterations were accompanied by a round-cell infiltration of the connective tissue, and later stages showed the new formation of connective tissue. The glomeruli exhibited acute and chronic changes. After intraperitoneal injections marked inflammatory reactions of the peritoneum were obtained.

The functional investigation of the rôle of the liver in the causation of the anaphylactic reaction has yielded some interesting and suggestive results as far as the dog is concerned. The liver is negligible for the production of an acute anaphylactic reaction in the sensitized guinea-pig, rabbit, and cat: the anaphylactic lung may be obtained after the liver and intestine are excluded by ligatures; the excised sensitized lung itself responds typically when ventilated and perfused with the protein used for sensitization (Dale, 33); in the rabbit the typical heart effect may be obtained when the central nervous system is destroyed and the thoracic aorta and inferior vena cava are clamped (Auer, 6); and in the cat a similar procedure does not prevent the production of cardiac irregularities and stoppage. (Schultz, 115.)

In the dog, however, the liver appears to play an important rôle both in sensitization and intoxication. Manwaring (83, 84) was the first to call attention to the fact that a removal of practically all the viscera, except
the liver, of a dog sensitized with horse serum does not prevent the occurrence of a pronounced drop in blood pressure associated with incoagulability of the blood when the animal is reinjected. Manwaring then excluded only the liver from the general circulation by ligating the vena cava above and below this organ, and maintained the circulation by placing T cannulae in the inferior vena cava and portal vein and leading the tubing to the external jugular vein; all the viscera remained in normal connection, therefore, until the ligatures were tied. The injection of hirudin was necessary in order to prevent clots. Four dogs out of seven showed no drop in blood pressure when the horse serum was injected intravenously after closing the ligature wires, but showed atypical slow drops in blood pressure when the ligatures were loosened. Manwaring also states that shock may usually be obtained if the ligatures are opened within three minutes after the injection; if the time interval, however, is five minutes or more no shock develops, but another injection now produces a drop in blood pressure.

Voegtlin and Bernheim corroborated Manwaring’s results and improved his technique by employing sensitized Eck-fistula dogs combined with a ligation of the portal vein near the hilus of the liver; in these dogs clamping of the hepatic artery would exclude the liver completely. After the hepatic artery was clamped the authors never obtained any drop in blood pressure when the horse serum was injected, but a drop developed when the clamp was removed.

Voegtlin and Bernheim also made the important observation that three of the Eck-fistula dogs which were sensitized after the operation failed to show any anaphylactic reaction on reinjection. This has been corroborated by Denecke. The latter investigator failed to obtain an anaphylactic reaction in eleven Eck-fistula dogs which had been sensitized by the intravenous injection of 1 c. c. egg-white and tested after 3 weeks by the intravenous injection of 10 c. c. egg-white; there were no gastro-intestinal symptoms, no leukopenia, and no drop in blood pressure (the latter was tested only in 2 cases). If, however, the Eck-fistula was established 3 weeks after sensitization with egg-white, then the reinjection caused vomiting, bloody diarrhea, and in the one instance tested the blood pressure dropped to 30 mm. Hg. The liver, therefore, seems to be necessary to obtain sensitization in the dog.

In a further series of experiments Denecke brought forward evidence that a relation apparently exists between the concentration of the foreign protein reaching the liver and the degree of sensitization. He observed severe effects, for example, when dogs with a reversed Eck-fistula (Eck-fistula dogs with the inferior vena cava ligated; all the blood of the lower half of the body therefore passes through the liver) were sensitized and later intoxicated by the injection into a vein of the hind foot. If, however, the reversed Eck-fistula dogs were sensitized by an intravenous
injection into the anterior part of the animal, and intoxicated after an appropriate interval, by an injection into a vein of the hind foot, only mild symptoms appeared. Denecke explains this result by assuming that a greater degree of sensitization occurs in those dogs where the egg-white reaches the liver in a less dilute state; in the reversed Eck-fistula dogs the protein would, of course, be less diluted before reaching the liver if the sensitizing dose were incorporated through a vein of the hind foot than if the injection were made into the anterior half of the animal. Some remarkable liver alterations have been noted by Hashimoto and Pick; these authors describe a doubling or even trebling of the non-coagulable nitrogen in the guinea-pig’s liver after mere sensitization by horse serum; they also observed that the livers of the same species obtained after acute anaphylactic death show only slight or no post mortem autolysis.

Urine.—Pfeiffer (94) reports that the urine of guinea-pigs which suffered a severe subacute anaphylactic reaction is toxic to normal animals of the same species. The intraperitoneal injection of 1 to 2 c. c. causes severe symptoms resembling those of anaphylaxis; subcutaneous injection of this urine causes necroses similar to Arthus’ phenomenon.

Blood and Lymph System.—Blood.—A number of changes occur in the chemical and physical behavior of the blood as well as in the blood-cell picture during the anaphylactic intoxication. The most striking alteration is the reduction or loss of coagulability, which is most pronounced in the dog, less in the rabbit, and least in the guinea-pig. If arterial blood is removed from the dog during the height of the anaphylactic reaction it remains uncoagulated for hours, or even days. (Biedl and Kraus, 19; Arthus, 4.) When a clot finally does form it is usually soft and does not retract as a normal clot does. As the coagulation proceeds so slowly the red corpuscles settle completely, leaving a clear supernatant plasma, which sometimes shows many fine floccules. The “buffy coat” is barely indicated. In the rabbit Arthus (3) observed that clotting was delayed from one-half to one hour, while normal rabbit’s blood clotted within 10 to 12 minutes. Both in the rabbit and dog, as these animals recover from the anaphylactic reaction, the blood gradually regains its property of coagulating. In the guinea-pig no well-marked delay in coagulation is demonstrable if the blood is taken immediately after acute death. If the guinea-pig does not succumb acutely, a delay in coagulation occurs. Sirenskij reports that the blood of guinea-pigs, sensitized with horse serum and reinjected intraperitoneally, examined 15 to 45 minutes after the toxic injection, showed a definite delay in coagulation (Brodie’s chamber); the delay was longest in protracted cases. The fibrin-ferment content diminished slowly after the reinjection, but was almost invariably largely reduced in amount after 45 minutes. No alteration in the Ca or Mg content was observed by Sirenskij, but the fibrinogen seemed to be decreased in amount after the anaphylactic reaction.
The diminished coagulability of the blood may be considered as a secondary effect of the reinjection, for De Waele (133) states that the parenteral injection of any foreign protein causes as a primary and immediate reaction of the organism a thromboplastic action and an antithrombin secretion, which latter is perhaps referable to the liver; the two phases, one aiding coagulation, the other delaying it, follow each other in a wave-like fashion. However, this may be, probably, every investigator has observed marked fluctuations in the non-coagulability of the anaphylactic blood, both in the dog and rabbit. There are no records that any one has ever observed a hastened clotting of the blood when an originally non-toxic protein was employed for reinjection. Such hastened clotting may occur: Auer (9) observed that one rabbit of a series of five which had been sensitized by repeated subcutaneous and intraperitoneal injections of horse serum died acutely on intravenous injection, while the other animals reacted to the same serum with moderate anaphylactic symptoms. Immediate autopsy of this animal showed that the heart was not beating and had stopped in diastole. The right auricle and ventricle were filled with a blood clot; the superior vena cava and its branches, the abdominal vena cava, and renal vein were full, round, and filled with a solid clot; the veins of the portal system, however, contained no clot, but fluid blood; the liver was dark and rich in fluid blood on section. The right ventricle showed the typical toughness of its endocardial surface to a marked degree. There was no pulmonary edema and no foam in the trachea.

It was mentioned before that the antithrombin was perhaps secreted by the liver; but it must be noted that the blood in the rabbit can show a typical reduction in coagulation when not only the liver, but all subdiaphragmatic structures, are excluded. Auer (6) reports that a sensitized rabbit whose aorta and inferior vena cava had been clamped above the diaphragm after destruction of the entire central nervous system, and kept alive by artificial respiration, showed marked differences after reinjection in the coagulability of the blood when taken above or below the clamp; above the blood did not coagulate during thirty minutes, while the blood below the clamp clotted firmly in fifteen minutes. The heart showed the alterations typical for the acute reaction in this animal. The liver thus cannot be the sole source of antithrombin in the anaphylactic rabbit.

Complement.—A large number of researches deal with the rôle the complement plays in the anaphylactic reaction, and this has been especially investigated by Friedberger (47). While in general the complement content of the blood sinks more or less during the anaphylactic reaction, this loss of complement does not go parallel with the severity of the anaphylactic reaction. The blood of a guinea-pig which dies acutely may show no, or only a slight, loss of complement (Sleeswijk). Loewit and Bayer (81) produced an intravital fixation of the complement in a sensitized guinea-pig by injecting an anticomplement serum intravenously. Al-
though the test showed no free complement in the blood, these animals reacted typically when reinjected with the protein used for sensitization. Nor do the interesting salt experiments of Friedberger, where the intravenous injection of 1 c. c. of saturated sodium chlorid solution prevents the anaphylactic reaction in the guinea-pig, demonstrate the necessity of the complement, although strong salt solutions do inhibit the fixation of complement and antibody, as Ehrlich has shown. It might be assumed, for example, that the salt inhibited the activity, but not the formation, of the substance which produces the anaphylactic reaction, a supposition which was strengthened when Ritz showed that salt solutions exhibited a similar protective action against peptone intoxication. The change in osmotic pressure, moreover, produced by the salt leads to dilution of the blood, and this might be a factor (Bornstein). The true reason was advanced by Dale (34), who demonstrated with the excised uterus of sensitized guinea-pigs as test object, that a small increase of tonicity from 0.9 per cent. to 1.1 per cent. in a solution bathing the preparation was sufficient to cause a strong reduction in the response of this muscle when the anaphylactic test was made. A rise in the concentration of the bath solution to 1.3 per cent. produced almost complete abolition of response to the antigen. That a much greater concentration is at least momentarily obtained by the injection of 1 c. c. of a saturated salt solution in a small guinea-pig is clear, and Dale calculates that this amount raises the sodium chlorid content of the blood, at least momentarily, to 3 per cent.

From the experiments quoted above it seems that the complement is not an essential factor in the anaphylactic reaction.

Changes in the Blood Picture.—During the anaphylactic reaction in the dog the leukocytes show a diminution in number. The leukopenia is due to a practical disappearance of the polymorphonuclear cells from the circulating blood, while the mononuclear forms and the blood platelets show an increase. As the animal recovers the polymorphous forms gradually increase and a leukocytosis develops (Biedl and Kraus, 22). Leukopenia occurs also in the rabbit and guinea-pig. This specific leukopenia was observed first during the serum disease and investigated by v. Pirquet and Schick (97), who state the number of leukocytes increases moderately during the period of incubation, but then sinks considerably during the appearance of the serum reaction. Here also the leukopenia is due almost entirely to the diminution in polymorphonuclear cells; the mononuclear forms show a slight relative increase. V. Pirquet and Schick call attention to the fact that the leukocyte curve during serum disease shows a strong resemblance to that observed in measles, small-pox, and vaccinia.

Leukopenia may be produced in rabbits by a single injection of horse serum. (V. Pirquet and Schick, 97.) The eosinophilic cells are not increased during the acute reaction in experimental anaphylaxis of the guinea-pig and dog, but occur in considerable numbers after a delayed
reaction. In addition to peripheral eosinophilia, Schlecht and Schwenker (113) obtained marked eosinophilia of the lung tissue and bronchi in guinea-pig, and the inflammatory edema of the subcutaneous tissue (Arthus' phenomenon) showed the exudate cells to be largely true eosinophiles. Eosinophiles in large numbers were also found in the submucosa of the gut of dogs who succumbed 11 to 18 hours after reinjection.

This eosinophilia is a true anaphylactic reaction, for Schlecht and Schwenker obtained no eosinophilia of the lungs after a single intraperitoneal injection of serum; nor did a single inhalation of sprayed serum lead to local eosinophilia of the lungs, but inhalation of serum by a sensitized pig caused typical eosinophilic infiltration of the lung tissue. Asphyxia or the intraperitoneal injection of Witte's pepton did not affect the eosinophiles.

In passive anaphylaxis no eosinophilia is observed. There is no relation between the degree of anaphylactic reaction and the degree of eosinophilia.

LYMPH.—Lymph of the dog, collected from the thoracic duct, is greatly increased in quantity during the anaphylactic reaction; at the same time the lymph, like the blood, becomes incoagulable (Calvary).

In the plasma and serum of guinea-pigs which died in the anaphylactic reaction H. and L. Hirschfeld demonstrated vasoconstricting substances when perfused through the Trendelenburg frog preparations. They are inclined to consider these substances protein cleavage products.

Nervous System.—Although the nervous system formerly occupied a prominent place, especially in theoretical discussions of anaphylaxis, the number of demonstrable functional or anatomical lesions is not great. Gay and Southard (55, 56) observed occasional hemorrhages in the brain, medulla, and spinal cord of guinea-pigs. The same authors also described lesions of the peripheral medullated sensory and motor nerves stained by the Marchi method. These were focal in type, in the myelin sheath, and especially at the node of Ranvier. The same authors noted an increased irritability of the vagus nerve in guinea-pigs sensitized with horse serum when horse serum was applied to that nerve. This increased irritability showed itself by marked respiratory symptoms; the application of physiological saline had no effect.

Yamanouchi, on the other hand, describes a reduction of sensitiveness when the cut sciatic nerves of rabbits sensitized with horse or bovine serum were bathed with the serum used for sensitization. The loss of irritability or conductivity (Yamanouchi does not state whether the faradic stimuli were applied at or above the site of the serum application) occurred within one minute after the cotton soaked in serum was applied. The reduction was marked: before the serum application, when only saline had been applied, 340 mm. coil distance gave a response; after the serum application a coil distance of 190 mm. was necessary. This loss, moreover, was specific;
application of horse serum to the nerve of a rabbit sensitized with bovine serum, and vice versa, had no effect.

The observations of Fröhlich may perhaps furnish the anatomical basis for Yamanouchi's results, although Fröhlich worked with frogs. The frogs had been sensitized by the injection of sheep or pig serum into a dorsal lymph sac. After 8 to 15 days they were curarized and the mesentery prepared for microscopical examination in vivo. Local application of the serum used for sensitization caused a marked local edema of the non-medullated nerve fibers in the mesentery, so that the nerves were often three times as thick as normal. This damage to the nerve was only observed in the neighborhood of the site of application; further away the nerves always showed a normal outline.

**Temperature Changes.**—In the subacute anaphylactic reaction the temperature sinks markedly, and in very mild cases this lowering of the temperature may be the only manifestation that an anaphylactic reaction has occurred. In acutely fatal reactions in the guinea-pig different animals behave differently and no drop in temperature may occur. Pfeiffer (93, 94), who discovered this "temperature drop," soon realized that the abrupt lowering of the temperature is not characteristic when considered by itself alone, for a large variety of substances may produce the same effect. By a strict adherence to a certain dosage, weight of the guinea-pig, and so forth, Pfeiffer (93), however, believes that a drop in temperature above 1.5° C. is conclusive evidence that an anaphylactic reaction has taken place.

In order to gain some insight into the causation of the drop of temperature the respiratory gaseous exchange has been examined. Both Scott and Loening observed in rabbits and guinea-pigs placed in a respiratory chamber that a non-fatal anaphylactic reaction causes a diminution in the \( \text{CO}_2 \) output and in the \( \text{O} \) consumption. Loening suggests that there is no increased dissipation of heat, but a definite diminution of heat production, for measures taken to prevent the loss of heat of the animal did not affect the result.

The temperature drop of Pfeiffer, which has also been observed in the rabbit and dog, is not the only temperature change which occurs in sensitized animals. Friedberger and his collaborators, especially Mita (49), observed that the temperature drop in sensitized guinea-pigs becomes less with a decrease in the dose employed for reinjection, and finally with a certain dose no temperature effects are obtained. If, however, this non-effective dose is still further decreased so that they are infinitesimal, Friedberger and Mita then observed rises in temperature. In normal guinea-pigs the injection of a foreign protein, as is well known, also causes fever, but Friedberger and Mita show that the quantity necessary for this effect is many thousands of times less in sensitized guinea-pigs than in normal ones. The sera employed by Friedberger and Mita were horse and sheep
séras, which were used as fresh as possible both for sensitization and reinjection. By a judicious variation in the amount of foreign protein injected, and in the interval between injections, Friedberger produced continuous, remittent, or intermittent fever in sensitized guinea-pigs. This protein fever he explains as the result of protein cleavage products which are formed by the body from the injected protein; this digestive capacity which the normal organism possesses is enormously increased in the sensitized organism because specific antibodies are present which facilitate the formation of these pyrogenic components from the protein molecule.

Vaughan also has independently produced in animals all the various types of fever which are met clinically by the injection of a toxic protein fraction. Both he and Friedberger give highly suggestive and stimulating applications of these facts in regard to the temperature reactions and causation of the acute infectious diseases.

Local Anaphylaxis.—Local reactions occur in the sensitized organism when the foreign protein is injected intracutaneously, subcutaneously into the conjunctiva or trachea. The ophthalmic-reaction of Wolff-Eisner and Calmette, and the skin reaction of v. Pirquet for tuberculosis belong to this class. The marked local reaction known as Arthus' phenomenon may serve as type, which has been described briefly on page 48. It may be added that Schlecht and Schwenker found the infiltrated cells of this local reaction to be largely eosinophiles.

When sensitized guinea-pigs are allowed to inhale a fine spray of the foreign protein Friedberger obtained pneumonia-like changes in the lung. Ishioka, with the same procedure, obtained only slight lung changes, but observed definite lesions when the foreign serum was injected into the trachea. The quantities injected were very small, 0.05-0.1 c. c. The majority of the guinea-pigs showed genuine pneumonias when killed. The pneumonia was lobar in type, though a whole lobe was rarely involved; the bronchi were not inflamed and the alveoli contained leukocytes, fibrin, and red corpuscles. All the lungs examined showed a more or less pronounced emphysema, which Ishioka considers an important factor in the production of the pneumonia.

CENTRAL OR PERIPHERAL CAUSATION OF THE ANAPHYLACTIC REACTION

In the preceding description of the main alterations which the anaphylactic reaction produces in the various animal species enough evidence has already been given to show that in many instances these alterations are of peripheral origin and are not dependent upon a reaction occurring in the cells of the central nervous system. Nevertheless, as the central nervous system was not absolutely excluded, and as reactions in the
nerve cell were formerly prominent in the explanation of anaphylactic phenomena, some of the experiments which definitely excluded the central nervous system may now be briefly reviewed.

Pearce and Eisenbrey (92) proved that the brain and medulla of the dog had nothing to do with the anaphylactic drop of blood pressure by obliterating all vascular connections between the head and trunk of a sensitized dog and maintaining an independent circulation through the head and neck by transfusion from the carotid artery of a normal animal. Under these conditions the injection of a foreign protein (horse serum) into the independent cerebral circulation of the sensitized animal caused only a slight, transient lowering of the blood pressure. When, however, the serum was injected into the trunk a typical persistent drop of blood pressure took place.

This fine experiment of Pearce and Eisenbrey shows absolutely that the centers of the medulla and brain, especially the central vasomotor mechanism, have no part in producing the drop in blood pressure. The same authors also demonstrated that after destruction of the cord and section of the vagosympathetic nerves a drop of blood pressure, nevertheless, results when the animal is reinjected. For the dog, therefore, it has been definitely established that the medulla and brain exert no causative effect upon the anaphylactic drop in blood pressure.

That the typical anaphylactic lung in the guinea-pig is due to peripheral causes, and is entirely independent of the central nervous system, was shown by Auer and Lewis and by Schürer and Střasman, who obtained the typical response after section of the vagi and destruction of the brain, medulla, and spinal cord. A still more striking proof was furnished by Dale (33), who produced the typical immobilization of the lung in the isolated organ after perfusion with the protein used for sensitization.

The cardiac changes which characterize the anaphylactic reaction in the rabbit were obtained by Auer (6) after section of the vagi and destruction of the cord, medulla, and basal brain; and typical cardiac anaphylaxis was described by Launoy in the excised heart of sensitized guinea-pigs after perfusion with the antigen. For these anaphylactic alterations the central nervous system is again not necessary.

That no central nervous system is necessary to produce the anaphylactic contraction of sensitized smooth muscle is clearly shown by the experiments of Schultz and Dale on excised loops of intestine and on the uterus.

The local anaphylactic reactions typified by Arthus' phenomenon are probably also produced independently of the central nervous system, though this has not yet been proved; it is difficult at least to conceive how the central nervous system could be the chief factor in this disturbance.

It must be observed that the experiments where the central nervous system was destroyed, or where the typical reaction was obtained with the excised organ, only show that the brain, medulla, and cord are not neces-
sary to obtain the typical result; they do not justify the inference that no reaction occurs in the central nervous axis. Rigid evidence for such a statement has so far been furnished only for the blood-pressure drop in the dog, where the higher nervous centers were maintained in a state of integrity by a cross circulation from a normal animal. (Pearce and Eisenbrey.)

There is, however, some evidence that the higher nervous centers may possibly be primarily affected in the anaphylactic reaction. The initial respiratory changes observable in the rabbit which sometimes occur before the blood pressure declines are perhaps due to a central effect. The initial rise in blood pressure in the same animal may perhaps also be caused by a stimulation of the vasomotor center. The respiratory symptoms in the dog and guinea-pig, however, are probably not due to a primary effect upon the nervous centers. In the dog they are best explained by an anemia of the higher centers which is secondary to the drop in blood pressure, and this also accounts for the stage of excitation and the following depression. The respiratory symptoms in the guinea-pig from beginning to end are very likely secondary to the asphyxia which begins as soon as the protein is reinjected intravenously.

The rise in temperature, nausea, and vomiting may possibly be due to primary central effects.

The diarrhea in dogs is probably largely peripheral and is caused by the congestion of the mucosa, the increased secretion of the pancreas, and especially by the strong contractions of the intestinal musculature.

Besredka’s (16) experiments on the protective action of ether anesthesia in the anaphylactic reaction of the guinea-pig do not demonstrate a central action of the anaphylactic reaction because ether causes a bronchodilatation, as Dixon and Brodie have shown, and this bronchodilatation probably neutralizes or reduces the bronchoconstrictor effect of the reination. Morphin, chloral-hydrate, and urethan also probably owe their effect to the same action on the bronchial tubes.

As another proof that the higher nerve centers are the seat of anaphylactic reactions Besredka and Steinhardt (18) advanced the great sensitiveness of sensitized guinea-pigs to intracerebral injections. This, however, is no rigid proof, for the results following such an injection may just as well be due to rapid absorption, as the brain is richly supplied with blood vessels.

The protective action which trephining exerts on the guinea-pig, according to Friedberger and Grüber, is difficult to explain, unless vascular shock and consequent poor absorption were produced by the operation.

It is therefore seen that the central nervous system on the whole seems to occupy a surprisingly subsidiary place as far as primary anaphylactic changes are concerned. That a large number of secondary reactions occur
in the brain and medulla as the result of peripheral anaphylactic changes is, of course, obvious.

**ANAPHYLACTIC MANIFESTATIONS IN MAN**

**Serum Disease.**—The best known example of anaphylaxis in man is the symptom-complex called serum disease by v. Pirquet and Schick. In a classical research these authors investigated the functional disturbances which occur in a percentage of cases after single or repeated injections of therapeutic sera in the human subject. The serum disease is characterized by fever, skin eruptions, swelling of the lymph glands, edema, leukopenia, and joint symptoms. The general condition, as a rule, is excellent.

The onset of the symptoms does not occur at once after the first injection in the great majority of cases, but only after a quite definite period of incubation, usually 8 to 12 days. The amount and character of the serum apparently exert no effect on the duration of incubation; nor is the incubation period referable to a delayed absorption for the antitoxic effects of the sera injected are exerted a few hours after injection. Moreover, quantities as large as 200 c. c. of serum leave no definite swelling 24 to 48 hours after a subcutaneous injection.

After the period of incubation fever and skin eruptions develop. The fever is one of the most constant symptoms, and may last from a few days to several weeks. It may be of a continuous or remittent type, and may reach 104° F. and over. The quantity of serum injected bears a definite relationship to the incidence of serum disease: after small amounts of serum, not more than 15 c. c., about 6 per cent. showed fever; but after the injection of 100 to 200 c. c. 85 per cent. of the cases showed the serum disease.

The skin eruptions present a great variety of forms and are mostly closely associated with the fever; they may be urticarial, scarlatinoid, morbillous, or polymorphous exanthems. Usually the first exanthem which appears belongs to the urticarial group. The first crop lasts a short time, but new ones may appear in other places for days. The exanthems usually appear first at the site of injection; the succeeding ones generally affect symmetrical parts of the body. The exanthems, like the fever, may last from a few days to several weeks.

Preceding the appearance of the eruptions the lymph glands draining the site of injection often become enlarged and tender. The enlargement increases and becomes general as soon as fever and skin eruptions develop. The glandular swelling decreases shortly before the general serum disease process abates, and is therefore of prognostic value.

During the incubation period the number of leukocytes is moderately
increased, but an abrupt diminution takes place on the appearance of
serum manifestations. The leukopenia, which is almost entirely due to a
diminution of the polymorphonuclear type, lasts only a few days, and
then disappears abruptly.

Joint symptoms are quite infrequent, but are very painful when pres-
et. They occur chiefly in the metacarpophalangeal, the wrist, and
knee joints, but examination reveals no objective alterations. V. Pir-
quet and Schick never observed any permanent disability as a result of
these joint symptoms. For treatment the authors advise cooling lotions;
the administration of salicylic acid preparations gave no relief.

Edema may be a pronounced symptom during the serum disease; its
location is similar to the edema of nephritic origin, first the face, then
the dependent parts of the body. As a rule there are no symptoms of
kidney irritation, and the albuminuria, when it does occur, never exceeds
0.25 per cent. This albuminuria, when present, is noted first during the
second and third week, and not immediately after the serum injection.
The edema persists throughout the course of the serum disease, but begins
to decrease shortly before the end of the disease. This decrease in edema
has the same prognostic value as the decrease in swelling of the lymph
glands; both indicate that the end of the serum disease is at hand. V.
Pirquet and Schick consider this edema as a primary symptom, and not
as a secondary effect of kidney congestion or insufficiency.

The mucous membranes are only exceptionally involved during the
serum disease, but in a number of cases a diffuse bronchitis and bloody
diarrhea were observed. A causal relationship between these disturbances
and serum disease v. Pirquet and Schick consider probable only for the
diarrhea. It will be remembered that diarrhea is a prominent feature in
the anaphylactic reaction of the dog.

Reinjections.—If a patient has been once subjected to the action of a
therapeutic serum, especially if large amounts were incorporated, his reac-
tion to a subsequent injection varies in a definite way.

I. After an interval of 12 to 40 days an immediate reaction occurs
which may be local or general, or both. Within 24 hours after the injec-
tion the local swelling increases markedly in size, and urticaria and fever
appear; the symptoms last only 1 to 2 days as a rule, but may be quite
severe. There is practically no incubation period. It is hardly necessary
to point out that the local edema following the injection corresponds to
Arthus’ phenomenon in the rabbit.

II. After an interval of 1.5 to 6 months an immediate and an ac-
celerated reaction may occur. The accelerated reaction is one where the
incubation period is shortened to 5 to 7 days. The symptoms are the same
as those observed after a first injection: fever, exanthems, edema, etc. The
accelerated reaction may also last only a single day, but, like the immediate
reaction, may be quite severe.
III. After an interval of more than 6 months only the accelerated reaction is observed as a rule.

The time intervals given above for the appearance of immediate and accelerated reactions must not be taken in a rigid sense, as many variations occur. Goodale, for example, observed an immediate (after 30 minutes) and an accelerated reaction (after 4 days) in a case which was reinjected subcutaneously seven years after the administration of the first dose. On first injection this individual showed serum disease after an incubation period of 18 days. Goodale’s case also illustrates the length of time that sensitization may be maintained in man.

The immediate reaction, local, as well as general, is sometimes obtained on first injection, but v. Pirquet and Schick consider the accelerated reaction as practically pathognomonic of the fact that the patient has been treated previously with serum. V. Böokay, however, observed a case where comparatively fresh serum (2 months old) produced an accelerated reaction in a child which was injected for the first time.

The frequency with which the serum disease occurs depends largely upon the amount of serum injected. Formerly, when 100 to 200 c. c. were injected, v. Pirquet and Schick observed the serum disease in 85 per cent. of the cases. With the reduction in quantity necessary to administer the proper amount of antitoxic units to 5 to 15 c. c. the percentage sank to about 6. This diminution has also been observed in the reinjected cases. Nemmsen collected 900 cases which had been injected twice, and 102 cases which had received 3 to 5 serum injections, nevertheless, only 42 (4 per cent.) developed a serum exanthem. Still more interesting was Nemmsen’s observation that not one of these 1,002 reinjected cases developed serious anaphylactic reactions.

An observation of v. Böokay seems to show that the character of the serum may play a rôle in the frequency with which serum exanthemata develop. In 1908 v. Böokay noted that in 19 out of 183 cases (10 per cent.) developed the serum disease, but in 1909 the number increased to 23.5 per cent. (43 out of 184). All the 1909 injections had been made with the serum from one horse, and v. Böokay concludes that the increased occurrence of serum disease was ascribable to some individual peculiarity of the horse which furnished all the serum.

Other Anaphylactic Manifestations in Man.—The serum disease, as characterized by the immediate and accelerated reactions in man, is not the only anaphylactic effect observable in man. Cases of collapse and even death, have been reported after the injections of small quantities of serum, though these accidents fortunately are rare. The symptoms observable under these conditions bear some resemblance to those observed in the lower animals, and it is quite probable that their causation is the same. A few examples may be given to illustrate this. V. Pirquet and Schick (97) report a case which was reinjected with 16 c. c. of serum 27
days after the first injection. Within 10 minutes the site of injection showed redness and urticaria; a short time later urticarial patches appeared scattered over the body. Fifteen to 20 minutes after the injection the boy began to vomit, his eyes rolled inward, the extremities became cyanotic, salivation occurred, and the pulse was no longer palpable. After the application of stimulants and warm packs the boy recovered.

This case probably suffered from a severe drop in blood pressure, which was caused either by a paralysis of the vasomotor endings of the gut, similar to that obtained in the anaphylactic dog, or by a weakening of the heart such as occurs in an anaphylactic rabbit, or by a combination of these two factors.

The first injection of horse serum has been followed in a number of instances by collapse and death, with symptoms which are very suggestive of those which occur in the dog, rabbit, and guinea-pig. Gillette has collected a number of cases from the literature where the injection of antitoxin serum caused collapse and death under symptoms which suggest the picture of acute serum anaphylaxis in the guinea-pig and rabbit. In this collection of thirty cases twenty-two gave a previous history of respiratory trouble, especially asthma. On injection some of them showed a remarkable dyspnea, and even convulsions, while the pulse remained full and regular. A picture of this type resembles the anaphylactic reaction in the guinea-pig. Moreover, in two cases the lungs were apparently larger than normal on autopsy. In other cases the injection produced a feeling of anxiety, depression, cyanosis, and complete collapse, associated with a feeble pulse. Cases of this type undoubtedly indicate disturbances of the heart and circulation, such as may be observed in the rabbit and dog during the anaphylactic reaction.

Disturbances of the gastro-intestinal canal have already been mentioned; v. Pirquet and Schick reported two cases in their monograph, and Gottstein called attention to a hemorrhagic enteritis which was observed a number of times on autopsy.

Reactions After Intraspinal Injections of Serum.—Especially severe, and sometimes fatal, cases have been reported after intraspinal injections of antitoxin serum, and these reactions have often been ascribed to anaphylaxis. Although anaphylactic reactions can easily be obtained from the spinal canal, as Besredka and Lissofsky have shown in the guinea-pig, nevertheless a study of some of the human cases, which are frequently quoted as examples in the literature even by Besredka (15), does not bring conviction that they are undoubtedly anaphylactic. To illustrate this statement the well-known report of Hutinel may be mentioned.

The paper of Hutinel, for example, reports four cases of death after the intraspinal injection of the Doper antitoxin serum, and protocols are given of three. Two of the cases died after an intraspinal injection of 30 c. c. given after intervals of 3 and 5 days. The intraspinal injections
before this were given daily. The arrangement in time of the injections
does not suggest that a high degree of sensitization could be produced.
The incubation period is exceedingly short, only a few days; moreover,
the daily injections ought to have produced the so-called immunity which
is obtained in guinea-pigs by the daily administration of massive doses
of serum. In the third case 150 c. c. of serum were injected in toto, 40
of them subcutaneously; serum disease developed after 17 days and lasted
8 days. Another intraspinal injection of 20 c. c. was administered 44 days
after the last one, but only a general urticaria without fever developed in
3 hours and disappeared in 24 (immediate reaction of v. Pirquet and
Schick). But another intraspinal injection of 30 c. c. given only 5 days
after the last one caused death. Here again the period of incubation is
too short for a high degree of sensitiveness; moreover, the patient should
still have been more or less refractory from the previous injection. The
doubt that anaphylaxis is the cause of death is strengthened still more by
the clinical symptoms and the speed with which they developed. All
developed symptoms shortly or immediately after the injection which are
observable after a rapid rise in intracranial pressure: hyperextension of
the body, with or without convulsions, and subsequent coma. Immediate
responses were also observed by Besredka (15) when serum was injected
intraspinally in guinea-pigs, while the anaphylactic symptoms appeared
only 1 to 5 minutes after the injection. In Huitinell's case III the symp-
toms are described more closely: the respiration was extremely slow and
irregular; the inspirations slow and noisy, the expirations short and fol-
lowed by long pauses. Pupillary and corneal reflexes were abolished;
the face was cold and pale; the extremities cyanotic. As the symptoms per-
sisted lumbar puncture was performed after five minutes and 30 c. c. with-
drawn "with ease." The respiration improved at once; the face gained
color, but the coma persisted and the patient died after 1½ hours. The
temperature remained normal.

In this last case also the symptoms were at least partly due to cerebral
pressure. In all these cases it appears unlikely that anaphylaxis caused
the symptoms; they are probably due to an increased pressure in the cen-
tral nervous system, a supposition which is strengthened by the fact that
the serum was apparently injected without first withdrawing an equal
bulk of spinal fluid.

The case reported by Grysez and Dupuisch probably belongs to the same
category. A patient received intraspinally 100 c. c. of Dopter and Flex-
ner serum given in 6 injections during 8 days. After 23 days another
injection was necessary. In order to avoid anaphylaxis the authors in-
jected 2 c. c. of Flexner serum intraspinally and waited 3 hours for de-
sensitization to establish itself. Then 40 c. c. of Flexner serum were
injected. After 30 c. c. were in, the head was retracted violently and
fibrillary contractions appeared; the patient was semi-comatose with ster-
torous respiration, dilated pupils, cyanotic face, and thready pulse. Nevertheless the injection was completed. The patient recovered swiftly as from a sleep, state the authors. In these cases of Hutinel and Grysez and Dupoich the dominant rôle attributed to anaphylaxis in the production of the symptoms is therefore at least open to question, and they should not be cited as undoubted proof. While intraspinal injections of serum undoubtedly may produce anaphylactic reactions the frequency of severe anaphylactic effects has probably been overestimated, and they can under no condition be considered a contraindication to the therapeutic use of the serum.

**Food Idiosyncrasies.**—There are numerous cases on record where the ingestion of certain protein foods, such as eggs, pork, milk, and sea food in general, produced marked reactions. At least some of these cases are true examples of anaphylaxis, for passive sensitization of guinea-pigs has been accomplished with the sera of some of these patients. These idiosyncrasies may be so marked that, for example, the application of egg-white on the skin or mucous membranes may produce a severe reaction. Sensitization in these cases was probably accomplished through an abnormally permeable respiratory or gastro-intestinal mucosa, or the tendency may have been inherited. The same explanation probably applies to those cases which react severely to the first injection of horse serum, and here also inhalation, ingestion, or heredity may explain the sensitized state.

**Hay Fever.**—This disease is also an example of anaphylaxis, and is caused by the proteins of various pollens. The disease does not develop before the fifth year, and may not occur until adult age. It is therefore probably acquired, and its acquisition is undoubtedly aided by an abnormal permeability of the nasal mucosa.

**Passive Anaphylaxis**

The injection of an animal with a foreign protein is not the only way in which sensitization can be produced. The sensitized state may also be established by injecting into a normal animal the blood or serum of an animal already sensitized. This important fact that the sensitized state is transferable from one animal to another was discovered by Gay and Southard and by Otto for foreign serum, and by Richet for toxalbumins. In active anaphylaxis, therefore, a reaction body or antibody is formed which carries the property of sensitizing against that protein to which it owes existence. Because a reaction body is formed in active sensitization the proteins which produce anaphylaxis are often called anaphylactogens, or antigens, thus classing the anaphylactic reaction with the other well-known immunity reactions.

The transfer of the sensitized state may be obtained, not only between animals of the same species (homologous sensitization), but also between
animals of different species (heterologous sensitization), provided that the animals employed are mammals, for the attempts to passively sensitize mammals from fowl, or vice versa, have failed. The animal employed most frequently for the production of the anaphylactic reaction body is the rabbit, and the test is usually made in the guinea-pig, because this animal is more readily passively sensitized than the rabbit or dog.

A refractory period is always present in the guinea-pig when the antiserum is injected first. After intraperitoneal injection in the guinea pig a 24-hour interval is necessary, but this period is shortened to 4 hours when the antiserum is injected intravenously. Reactions are, however, obtained in the guinea-pig when antiserum-antigen mixtures are injected intravenously. Since the refractory period is always present when the two components are injected separately, it is quite possible that the reaction obtained with the mixture is not one of passive anaphylaxis, but is perhaps due to the formation of a poison by the interaction of antigen and antiserum; this supposition is strengthened by the observations that the simultaneous, but separate, injection of antiserum and antigen (each into a jugular vein), or the injection immediately after the mixture, as a rule, produces no reaction.

The necessity of the interval between the injection of antibody and antigen is explained by the assumption that the antibody undergoes certain changes in the guinea-pig or enters into certain relations with organs before it is able to react with the antigen and produce the disease.

Conditions are somewhat different in passive anaphylaxis of the rabbit, for here no interval is necessary between the injection of antibody and antigen; the animal is immediately sensitized after the injection of the antiserum, and reacts even more powerfully when the antigen is injected at once than if a 24-hour interval is allowed to elapse. Moreover, it has been shown that a specific local edema may be obtained in the rabbit when the antigen is injected first and the antiserum after 24 hours; in the guinea-pig this procedure prevents passive sensitization.

The symptoms produced when passively sensitized animals are injected with the appropriate antigen are identical with those obtained during active anaphylaxis, and experimental analysis has established the same alterations in passive as in active anaphylaxis.

The anaphylactic reaction body has not been demonstrated in the blood of guinea-pigs before the animal itself has been sensitized by the foreign protein. Nor can it be detected in the blood during, and for some time after, the anaphylactic reaction. It has, however, been obtained later in the antianaphylactic stage, and may produce passive sensitization while the animal furnishing the antibody is still refractory to another injection of the antigen.

It is interesting to note that free antibodies cannot be detected in the blood after a certain time, although the animal is still sensitized. This is
probably to be explained by the assumption that the antibodies remain sessile and do not leave the cells forming them.

The length of time passive sensitization persists is only a few weeks; a test made after 15 days is, as a rule, negative.

Much time and labor has been spent in the endeavor to identify the anaphylactic reaction body with precipitin, but the outcome has not been a decisive answer for or against this view.

Passive sensitization can also be studied in the excised organ. Dale (33) has demonstrated that the uterus of a normal guinea-pig, when perfused for five hours with a 20 per cent. solution of anti-horse serum from guinea-pigs, followed by a perfusion of 500 c. c. Ringer solution, gave a typical tetanus when bathed in a 0.2 per cent. solution of horse serum. After relaxation and thorough washing of the organ with Ringer solution the renewed application of horse serum had no effect; the uterus was antianaphylactic. Dale was also able to resensitize the uterus of an actively sensitized guinea-pig after the preparation had once responded and was demonstrably antianaphylactic. In this case mere bathing, not perfusion, for three hours in a 10 per cent. solution of sensitive guinea-pig serum was sufficient to restore sensitization, and the preparation now responded typically when normal horse serum was added to the bath solution. As mere bathing in the antibody did not sensitize a normal uterus, Dale suggests that the cells which have once held antibodies take them up again more readily than normal muscle cells.

ANTIANAPHYLAXIS

After a sensitized animal has recovered from the anaphylactic reaction it becomes refractory to another injection of the same protein. This refractory state was first observed by Otto and Rosenau and Anderson; Besredka and Steinhardt named this state antianaphylaxis. A relatively short time only is necessary to bring on this refractory state, and its length depends upon the method chosen for the incorporation of the protein: after intraperitoneal injection 1 to 2 hours are necessary; after intravenous injection the desensitization occurs almost immediately; the longest time interval is necessary after subcutaneous injection. This rapid development of antianaphylaxis renders it possible to give large amounts of the antigen to a sensitive animal without producing symptoms, provided that the antigen is injected repeatedly in small amounts (Besredka, 15), or is infused intravenously at very slow speed (Friedberger and Mita). While Friedberger and Mita’s procedure protected as a rule only against ten fatal doses (time consumed during the injection was 50 to 60 minutes), Besredka has been able to protect against more than 200 fatal doses of the antigen. The procedure of Besredka (15) is as follows: In actively or
passively sensitized guinea-pigs, where the fatal dose is known, a fraction of this dose is injected subcutaneously, intraperitoneally, or intravenously. This dose vaccinates against one or two fatal doses within four hours if the vaccinating dose was administered subcutaneously, or within five minutes if the vaccination was intravenous. Repeated injections of this type gradually raise the tolerance to a high level. For example, in guinea-pigs sensitized with egg-albumen 1/500 c. c. intravenously killed in four minutes. In one animal of this series 1/2000 c. c. was injected intravenously with no reaction; after 10 minutes 1/500 c. c., the fatal dose, was injected with no effect; after 10 more minutes 1/50 c. c. was tolerated perfectly; ten minutes later 1/5 c. c. (100 fatal doses) caused no reaction; somewhat later 2 c. c. of undiluted egg-albumen were injected into the jugular vein. This injection of 1,000 fatal doses gave symptoms, but the animal recovered rapidly.

On the basis of these results Besredka (15) does not hesitate to give explicit directions to the physician how to proceed when it is necessary to inject serum intraspinally in order to avoid anaphylactic complications, for Besredka (15) mentions ten cases of death which he attributes entirely to anaphylaxis.

Antianaphylaxis occurs in the rabbit, dog; and doubtless in man, as well as in the guinea-pig, although differences exist between the species. The duration of the antianaphylactic state is very short in the rabbit, and lasts only a few days (Scott). Guinea-pigs, however, which have been injected intraperitoneally repeatedly with large doses of protein may remain antianaphylactic for long periods of time, although their blood shows the presence of antibodies. Rosenau and Anderson have produced an antianaphylaxis in this way which lasted for months. This procedure has been called an immunization by some authors, but it has been shown by Weil (136, 137, 138) that it is really a state of latent hypersensitiveness. Weil proved that the so-called immune guinea-pigs prepared by massive injections are really hypersensitive, and will succumb provided that a sufficiently large dose of the antigen is injected intravenously. Their refractoriness, according to Weil, is due to the fact that the sessile antibodies of the body cells are protected by the large amount of circulating antibodies. Other important facts regarding the production of antianaphylaxis were contributed by the same author. Weil showed experimentally that guinea-pigs sensitized with fractional doses of antigen can be desensitized or rendered antianaphylactic with small doses, while, after sensitization with large doses, large amounts of antigen are necessary to accomplish this purpose. The reason is that the number of antibodies formed stands apparently in some relation to the amount of antigen used for sensitization: after fractional doses the amount is small; after large doses the amount of antibodies present is much greater. Experimentally, therefore, unless the fatal dose or size of the sensitizing dose is known, antianaphylaxis can
only be produced by a slow process of graded doses such as Besredka employs, without any knowledge of when the desensitization is complete. This is a point of great importance in the practical application of Besredka's methods in the human subject, and Weil is justified in warning not to expect in man the striking results Besredka obtained in guinea pigs.

Neutralization of the anaphylactic antibody is not the only method of producing a refractory state. It may also be established by the injection of a number of other substances, for example, Witte peptone, as Biedl and Kraus (22) have shown in the dog. If a dog, sensitized with horse serum, is injected with peptone, the dog after recovery from this injection, does not react to a horse serum injection; it is thus in an aniantianaphylactic state. This non-specific aniantianaphylaxis, however, is not of high degree, nor does it last a long time. The differentiation between non-specific and specific aniantianaphylaxis has been especially investigated by Friedberger (52) and his collaborators.

Antianaphylaxis can also be obtained in the excised organ, as Launoy (74) has shown for the guinea-pig's heart, and Dale (33) for the excised guinea-pig's uterus.

PREVENTION OF THE ANAPHYLACTIC REACTION

Lower Animals.—The best procedure is probably Besredka's method of desensitization by a series of graded doses of antigen, and his procedure has been described in the section on antianaphylaxis. In addition to this method a number of different substances may be mentioned whose administration has abolished, reduced, or prevented the characteristic reactions in the animals used for the experimental investigation of anaphylaxis.

(1) Sodium Chlorid.—Friedberger and Hartoch have protected guinea-pigs by injecting about 1 c. c. of a saturated sodium chlorid solution intravenously before the antigen was given. The protective action is probably due to a reduced irritability of the smooth muscles which the increased toxicity of the blood causes. (Dale, 34.)

(2) Barium Chlorid.—Biedl and Kraus raised the blood pressure in the anaphylactic dog by the intravenous injection of 50 to 100 mg. of BaCl₂. A previous injection of the salt even prevented all anaphylactic symptoms in a sensitized dog.

(3) Peptone.—Biedl and Kraus observed that sensitized dogs, after recovery from the peptone shock (approximately 0.25 to 0.5 gm. per kilo intravenously), are immune to a subsequent injection of the antigen.

(4) Ether Narcosis.—Besredka recommended this procedure, and obtained good results in guinea-pigs. The protective action, which is not great, according to other observers, seems to be entirely due to a reduction in irritability of the bronchial muscles. In dogs vomiting is abolished by
ether narcosis, but the characteristic drop in blood pressure occurs promptly, with no sign of any diminution.

(5) **Atropin.**—This alkaloid was recommended for use in the guinea-pig, because it is the direct antagonist of the death-producing effect exerted in acute anaphylaxis in this animal, for atropin relaxes the bronchial muscles. The dose is 1 to 5 mg. intravenously, depending upon the severity of the reaction. A prophylactic dose of 2 to 10 mg. may be given subcutaneously. The protection is not absolute, but against a minimal lethal dose it protects in 70 per cent. of the cases. (Auer, 7.) It is only indicated in respiratory effects of the asthmatic type.

(6) **Urethan. Adrenalin.**—Both of these substances have a relaxing effect upon the bronchial muscles, as Dixon and Brodie showed for urethan, and Januschke and Pollak for adrenalin. Anderson and Schultz were able to save 66 per cent. of their guinea-pigs by combining these two drugs with chloral hydrate and giving artificial respiration with oxygen gas.

(7) **Chloral Hydrate.**—The action of chloral hydrate was investigated especially by Banzhaf and Famulener. These authors saved 75 per cent. of highly sensitized guinea-pigs by injecting about 75 mg. of a chloral hydrate solution (10 per cent.) intramuscularly 20 to 30 minutes before the intraperitoneal incorporation of the foreign protein (horse serum). The dose given is for a 250-gram guinea-pig. The drug may also be administered by intracardial injection; 30 mg. per 275 to 300 gm. of weight, repeated after 2 to 4 minutes. This procedure protected 75 per cent. of the sensitized animals from an intracardiac injection of the horse serum.

**Man.**—Before discussing the methods which are available for the production or treatment of severe anaphylactic reactions in man a few general remarks must be made. It has already been shown that, while reactions do occur, they are by no means common, and their frequency can surely be decreased if certain precautions are observed.

No therapeutic serum should be administered without strict indication; it should never be given "for good luck."

The serum should not be fresh. Fresh serum is in itself toxic. According to Boehmcke, it would appear that the reluctance of physicians to inject older sera is not well founded. Boehmcke found no diminution in the antitoxic value of aged diphtheria antitoxin after ten years, provided that the serum was protected from light and heat. Even when kept at a temperature of 37° C. for five months the serum showed only a slight loss.

A purified serum should be used when possible. The diminution in the amount of serum proteins necessary to produce results, for example with diphtheria antitoxin, has decreased the occurrence of the serum disease considerably.

Intravenous injections of therapeutic sera should only be given when
the patient's condition absolutely demands it. As a routine practice it is undoubtedly more dangerous than the subcutaneous injection, for laboratory experience has shown conclusively that highly sensitized guinea-pigs easily recover from a subcutaneous dose, a fraction of which would kill if given intravenously. It must be noted, however, that Park has observed about 300 cases where 5 to 7 c. c. of antitoxic serum were injected once or repeatedly without any serious symptoms. After larger intravenous injections of antistreptococcic serum (100 to 200 c. c.) the same observer noted a serious collapse but once in a sensitized case.

Caution must be exercised when it becomes necessary to administer a therapeutic serum to patients who have chronic respiratory troubles, especially asthma, or who have been injected previously with horse serum. With asthma cases desensitization ought to be attempted according to Besredka's methods.

In subjects who have already been injected with horse serum the danger is apparently not so great, though severe reactions do occur (Netter, Darling, and others). Nemmser collected the histories of 1,002 cases, of which 900 had received two injections, and 102 three to five injections of diphtheritic antitoxin, and failed to find any record of a severe anaphylactic reaction. Moreover, fever and exanthems developed only in 42 patients. The results are probably partly due to the small dose of serum employed, which varied between 6 to 10 c. c. for each injection.

When therapeutic sera are injected intraspinally (antimeningitis serum) a bulk of spinal fluid equal to the amount of serum to be injected should first be removed. It is more than probable that at least some of the cases reported by various observers, where convulsions and collapse occurred immediately after the injection, were due to pressure rather than to anaphylaxis. In experienced hands, moreover, the occurrence of severe symptoms is quite rare (Park).

It must in general be strongly emphasized that all the dangers incident to the warranted exhibition of therapeutic sera are almost negligible in comparison to the dangers of the untreated disease.

**Besredka's Methods**

Besredka (15) has described the following procedures especially for intraspinal injections of sera, when the patient has been sensitized by previous administrations of serum; in practice, however, he advises that every patient be considered as possibly sensitized.

If the diagnosis of intraspinal meningitis is undoubted 2 c. c. of the serum is injected intraspinally. After at least two hours the final dose of 20 to 30 c. c. is injected.

If the case is very urgent then the intravenous method of desensitization is recommended, 1 c. c. of a 10 per cent. solution of serum being in-
jected intravenously; after 4 minutes 3 c. c. more; 10 minutes later 10 c. c. are injected; after 2 more minutes 25 c. c. of the dilution are infused. Four minutes later the patient is desensitized, according to Besredka, and is able to endure 10 to 30 c. c. of undiluted serum either intravenously or intraspinally.

If the diagnosis of meningitis is doubtful Besredka advises, nevertheless, to inject the enormous dose of 10 to 20 c. c. of serum subcutaneously for vaccinating purposes, so that the next day the patient may, if necessary, receive 20 to 25 c. c. intraspinally.

It seems quite certain that these vaccinating doses advised by Besredka for the human being are too great. Netter (88) noted collapse after a subcutaneous vaccinating injection of 2 c. c. serum in a child which had been injected twice before, the intervals being 29 and 14 days, and Netter in consequence recommends that much smaller quantities be used for vaccinating purposes, for example, 0.1 to 0.01 c. c. This procedure would surely be safer, and its efficacy has been demonstrated in the guinea-pig. In this connection the warning of Weil (136) should be remembered, that a safe desensitizing dose can only be determined when the minimal lethal dose is known; a fraction of this dose could then be used with certainty as the first dose in the desensitization process. The minimum lethal dose is, of course, never known in the human subject, and this fact is therefore another strong argument for starting the vaccination process with extremely small quantities.

After severe anaphylactic symptoms have set in the treatment is more or less symptomatic. If the respiratory symptoms are of an asthmatic type atropin is indicated to relax the bronchial muscles. Adrenalin also relaxes the bronchial muscles (Januschke and Pollak), and besides delays absorption (Meltzer and Auer), thus facilitating desensitization.

If the blood pressure is low adrenalin may be given, although Biedl and Kraus' results in the dog were not encouraging. BaCl₂ is very toxic, but perhaps could be employed cautiously in cases of extreme and persistent low blood pressure. Biedl and Kraus obtained gratifying results in anaphylactic dogs with this drug.

For cardiac weakness and failure, which possibly also occur in the severe types of anaphylactic reaction in man, no treatment has been described. Digitalis preparations, if employed, must be used with caution, for Auer has observed that they apparently hasten cardiac death in the anaphylactic rabbit.

The treatment of serum disease is preventive and symptomatic. The prophylactic treatment is to use as small a quantity of serum as possible, and this has diminished the incidence of serum disease after antituberculous serum considerably. The symptomatic treatment, according to v. Pirquet and Schick, is as follows:
URTICARIA: 1 to 2 per cent. salicylic acid or 1 per cent. menthol in alcohol, or 1 per cent. menthol salve.

Fever: wet packs; no antipyretics.

Arthritis: salicylic acid preparations were found useless; baths and local applications.

Diarrhea: attention to diet and the ordinary treatment. Edema and albuminuria cannot be prevented by any known means.

CRITERIA OF ANAPHYLAXIS

It will be observed from the description of the anaphylactic reaction in the dog, rabbit, and guinea-pig that there is no single fundamental type of anaphylaxis which is available for purposes of comparison, nor is there even a single symptom which appears with equal intensity in the three species: for example, the lung immobilization is found practically only in the guinea-pig, and even there only after acute death; it does not occur in the rabbit, and only exceptionally in the dog; the characteristic abrupt drop in blood pressure is observed only in the dog, and the drop observable in rabbits and guinea-pigs has a different character; the coagulability of the blood may be lost in the dog, strongly reduced in the rabbit, and only slightly decreased in the guinea-pig; vomiting is common in the dog, but does not occur at all in the rabbit or guinea-pig; and so on, through all the symptoms ever described in experimental anaphylaxis. This varying intensity of effect of the anaphylactic reaction upon the different systems of organs in the dog, rabbit, and guinea-pig must be clearly kept in mind, for in anaphylaxis, as in every other reaction studied in vivo, each animal species must be measured with its own yard stick. At least some of the confusion in the literature of anaphylaxis is directly traceable to failure to realize this. The main factor which caused this error was the desire to unify, to standardize the anaphylactic reaction for all species of animals. This can probably be done, even with the evidence available at present, but the value of this standard for the discovery of new facts is doubtful.

It must also be remembered that none of the functional and anatomical changes which occur during the anaphylactic reaction in any animal are by themselves alone diagnostic of the anaphylactic state. All these changes, which have been described in some detail in the preceding pages, do not permit the diagnosis of anaphylaxis, unless they have been obtained on reinjection of some foreign protein. In other words, the functional and anatomical changes themselves are not characteristic of anaphylaxis, but the procedure of obtaining them is characteristic. What this procedure is has been described; the animal must first be sensitized by the incorporation of a foreign protein; after a period of incubation the reincorporation of the same protein must produce symptoms which were not present when
the animal was first injected. For passive anaphylaxis, at least in the
guinea-pig, the same three stages are observable. This procedure is the
essence of the symptom-complex of anaphylaxis, and only by its means
were Theobald Smith, Otto, and Rosenau and Anderson enabled to dif-
ferentiate it from similar intoxications caused by a variety of means. The
diagnostic value of the tuberculin test depends on this conception; if no
response is obtained after tuberculin it is assumed that that subject has not
been sensitized by tuberculosis proteins.

The anatomical and functional changes which the different animal
species, especially the guinea-pig, present during the anaphylactic reaction
may be produced by a large variety of different substances. For example,
toxic normal sera; immune sera; fresh defibrinated blood; Witte peptone;
urines from normal, anaphylactic, or scalded animals; protein cleavage
products; products of putrefaction; saponin, potassium cyanid; bacterial
extracts and many other substances may give a clinical and anatomical
picture, when injected into guinea-pigs, which differs but slightly from
that obtained on reinjection of a foreign protein in a sensitized animal.
Since so many different substances produce more or less the same result, it
is clear that great caution is necessary as soon as any one of them is indi-
cated as the cause of experimental anaphylaxis, for it is obvious that this
statement can only be an inference based on identity of action. That this
inference is not justified is clearly shown when one considers that an
identity of functional response to various causes does not prove that these
various causes are identical; although saponin and Witte peptone may pro-
duce practically the same lung picture in the guinea-pig, it cannot be con-
cluded from this observation that saponin and Witte peptone are identical
chemically, though they may be functionally identical in certain reactions.

In order to differentiate between those substances which produce
changes similar to, or even identical with, those obtained after the reinjec-
tion of a sensitized animal Auer (8) and Loewit (81) have suggested that
the term "anaphylactoid" be applied to the alterations resembling the ana-
phylactic types of reaction, but which are obtained on first injection into
a normal non-sensitized animal.

Some of the anaphylactoid phenomena demand further consideration.

ANAPHYLACTOID PHENOMENA

It has already been pointed out that a large number of chemically
different substances, when injected into an organism, produce at once
symptoms which resemble those noted during the anaphylactic reaction.
Such substances are found among the cleavage products of proteins, and
have been investigated especially by Vaughan, Schittenhelm and Weich-
ardt, Biedl and Kraus, and many others. The important researches of
Vaughan showed that all proteins can be split into a toxic and a non-toxic
constituent by boiling for several hours in a 2 per cent. solution of sodium hydrate in absolute alcohol. The toxic portion is alcohol soluble, the non-toxic fraction is insoluble. With the toxic fraction Vaughan and his collaborators were able to produce on first injection in guinea-pigs the symptoms and anatomical signs which are observable in the anaphylactic reaction of this animal. When injected into dogs Edmunds observed in general the same symptoms which acute anaphylaxis calls forth in this animal. The toxic fraction does not sensitize, but the non-toxic moiety can sensitize against the whole protein molecule, but not against itself.

Schittenhelm and his collaborators examined the protein cleavage products separately, and demonstrated that a number of different poisons are formed which individually often show certain resemblances in their physiological effect to the anaphylactic reaction; they observed a drop in blood pressure, leukopenia, diminished coagulability, and, in the guinea-pig, an immobilization of the lung.

Biedl and Kraus (22) injected Witte’s peptone into dogs and guinea-pigs, and obtained in both animals effects which they considered identical with those observed in true anaphylaxis in these animals. They conclude the anaphylactic intoxication is caused by a poison which is to be considered physiologically identical with the active constituent of Witte’s peptone.

In a large series of papers Friedberger and his collaborators have attempted to prove that a toxic mixture produced in vitro by the action of fresh guinea-pig serum upon specific precipitates (immune serum and antigen) is the true anaphylactic poison, because it produces the typical symptoms when injected into normal guinea-pigs, and because this toxic material, or “anaphylatoxin,” is formed from the same constituents whose interaction in the body apparently causes the anaphylactic intoxication. It is impossible here to survey the enormous literature which Friedberger’s anaphylatoxin has called forth, and for an adequate critical presentation of this question the reader is referred to the general review of Doerr (38).

In general it may be said that Friedberger’s anaphylatoxin theory is a modified form of the protein cleavage theory, for the “anaphylatoxin” is said to be split from the antigen by a process of digestion in which the complement and immune body play essential roles.

Among the anaphylactoid phenomena the so-called drug idiosyncrasies must also be placed, at least for the present. No definite evidence has yet been advanced that crystalloid substances produce the formation of an antibody of the type of the anaphylactic reaction body. For the large literature on this subject see Doerr’s review.

**THEORIES OF ANAPHYLAXIS**

As soon as the striking phenomena of anaphylaxis were carefully investigated a number of theories arose to explain their causation. A de-
tailed consideration of these theories is beyond the scope of this article, and a brief consideration of the leading conception will be given.

Practically all investigators consider the symptoms of anaphylaxis as due to an intoxication, to a poisoning of the tissue cells. This poison was thought to be formed either by the union of the antibody and antigen alone, or this combination of antibody-antigen was activated by the complement, and now, by a process of parenteral digestion, toxic cleavage products were formed from the antigen which produced the symptoms of anaphylaxis. This conception of an etiological relationship between anaphylaxis and protein cleavage products is the leading one at the present time, although, as Doerr points out in his excellent review, the most intensive work has not so far been able to establish the following three fundamental points: (1) the determination of the mother substance whose cleavage furnishes the poison; it is not known whether the injected antigen or the body proteins, or both, furnish these hypothetical cleavage products (Zunz); (2) the structure and properties of this poison, or poisons; (3) the proof that these products are formed during the acute anaphylactic reaction; the anaphylactic lung of the guinea-pig, for example, where these cleavage products must be present, according to hypothesis, showed no increase in the content of albumoses, peptones, or amino-acids, as determined by the method of Van Slyke (Auer and Van Slyke). Obviously these objections do not invalidate the parenteral digestion theory of anaphylaxis; it still remains the most attractive explanation yet devised, nevertheless the existence of these objections must be clearly kept in mind, for they show that the theory is not yet firmly established.

The parenteral digestion theory of anaphylaxis was first formulated on the basis of clean-cut experiments by Vaughan, and his exposition and development of it by laboratory work has enriched knowledge with many important facts and aided the comprehension of confusing phenomena. Vaughan's theory is briefly as follows: The introduction of a foreign protein into the tissues or circulating juices of an animal develops in that animal a proteolytic ferment which is specific for the protein injected. This specific ferment remains in the cells of the animal as a zymogen, and is activated when the same protein is again injected. A sensitized animal is thus one whose cells are rich in a specific proteolytic zymogen; moreover, each foreign protein has its predilection tissue, where it is largely deposited, whose cells it especially sensitizes, and where it is disrupted. As all proteins are conceived to be composed of a toxic and a non-toxic fraction, and as the second injection of the foreign protein activates the specific zymogen, the active ferment is liberated, splits the foreign protein, and the freed toxic component now produces the symptoms of anaphylaxis. The first injection of the foreign protein produces no toxic symptoms, because there is no specific ferment present, and the non-specific ferment present split the foreign protein so slowly that at no one time is a sufficient amount
of poison liberated to produce the ordinary symptoms of anaphylaxis.

Antianaphylaxis, according to Vaughan, is due largely to the quanti-
tative disproportion between the small amount of specific ferment now
available and the foreign protein; for the anaphylactie reaction uses up a
large part of the ferment, and the remainder can produce too little poison
to exert any effect. Passive anaphylaxis is explained as the transfer of the
specific proteolytic zymogen, the antibody in terms of Ehrlich's theory,
from a sensitized animal to a normal one.

This is the bare skeleton of Vaughan's theory, a conception which, in
various forms, has been more fruitful of results than any other theory of
anaphylaxis formulated thus far. Whether time will demonstrate its truth
or not matters little; it has already fulfilled the main function of a theory:
it has stimulated research and produced an abundance of new facts.

REFERENCES

For a complete bibliography of anaphylaxis the reader is referred to Robert
Doerr. Allergie und Anaphylaxie, Kölle-Wassermann's Handb.
d. patho. Mikroor. 2d Ed., 1913, ii, 947-1154. Also by the
same author—Neuere Ergebnisse d. Anaphylaxiforschung in
Ergebnisse d. Immunitätsforschung, Exp. Therap., Bakt. u.
Hyg., 1914; i, 257.

Another review of anaphylaxis has been given by Erich Seligmann. Ana-
phylaxie, Oppenheimer's Handb. d. Biochemie des Menschen und

The following are the most important monographs which deal with
anaphylaxis:

E. Friedberger. Die Anaphylaxie. Fortschritte der deutsch. Klin., 1911,
ii, 619-726.

A. Biedl and R. Kraus. Die experimentelle Analyse der anaphylaktischen
I, 1911, 255-290.

V. C. Vaughan, V. C. Vaughan, Jr., and J. W. Vaughan. Protein Split
Products in Relation to Immunity and Disease. 1913, Lea and
Febiger, pp. xii, 476.

Biol. and Med., 1910, vii, 32.

de physiol., 1909, vii, 471-526. This article contains the notes
first published in the Compt. rend. de la soc. de biol., 1903-1906.

3. ———. (2me mémoire) ibid., 1910, ix, 156-178.

4. ———. La séro-anaphylaxie du chien. Ibid., 1910, ix, 179-203.

5. Auer, J. The Effect of Vagus Section upon Anaphylaxis in
REFERENCES


9. ——. Unpublished work.


16. ——. Comment empêcher l'anaphylaxie. Compt. rend. de la soc. de biol., 1907, lxii, 1053.


THE FUNCTIONAL ANALYSIS OF ANAPHYLAXIS

34. ——. The Effect of Varying Tonicity on the Anaphylactic and Other Reactions of Plain Muscle. Ibid., 1913, iv, 517.
35. Darling, S. T. Two Cases of Anaphylactic Serum Disease Over Six Years After the Primary Injection of Horse Serum (Yersin’s Antistep Serum). Arch. of Int. Med., 1912, x, 440.
44. Eisenbrey, A. B., and Pearce, R. M. A Study of the Action of the
REFERENCES


47. Friedberger, E. Die Anaphylaxie. Fortschritte der deutschen Klinik, 1911, ii, 619; see also recent volumes of the Zeitsch. f. Immun.


74. Launoy, L. Compt. rend. de la soc. de biol., 1912, lxxii, 403; also ibid., 815.
77. Lewis, T., and Matheson, G. C. Auriculoventricular Heart-block as a Result of Asphyxia. Heart, 1910, ii, 47.
REFERENCES

80. Loewit, M. Die anaphylaktische und anaphylaktoide Vergiftung beim Meerscheinchen. Ibid., 1913, lxiii, 1.

81. —— and Bayer, G. Die Bedeutung des Komplementes für den akuten Shock bei der aktiven Anaphylaxie. Ibid., 1912, lxix, 315.


95. Pfeiffer, H. Weitere Beiträge zur Kenntniss der Uberempfind-


99. ——. De la substance anaphylactisante ou toxogénine. Ibid., 1908, lxiv, 846.


101. ——. Anaphylaxis. Translated by J. Murray Bligh, 1913.


REFERENCES


116. ——. The Reaction of Smooth Muscle of the Guinea Pig Sensitized with Horse Serum. Jour. of Pharm. and Exp. Ther., 1910, i, 549.

117. ——. Physiological Studies in Anaphylaxis. II. Reaction of Smooth Muscle from Guinea Pigs Rendered Tolerant to Large Doses of Serum. Ibid., 1910, ii, 221.


125. Smith, Theobald. Quoted from Lewis and Otto.


132. Voegtl lin, C., and Bernheim, B. M. Jour. of Pharm. and Exp. Ther., 1911, ii, 507.


137. ——. Desensitization; Its Theoretical and Practical Significance. Ibid., 1913, xxix, 233 (n. s. xxiv).


141. ——. Anaphylaxie und wachsartige Degeneration der Muskeln. Centralblatt für allgemeine Pathologie, 1912, xxiii, 945.


CHAPTER III

IMMUNOLOGICAL REACTIONS IN DIAGNOSIS

GEORGE F. DICK

Infections are followed by certain changes from the normal in the behavior of the infected individual toward the specific agent of the infection. Thus Jenner as early as the latter part of the eighteenth century made the observation that, when persons who had passed through an attack of smallpox or cow-pox were inoculated with variolous matter, an inflammation soon occurred at the site of inoculation. After persisting a short time this inflammatory reaction subsided without the more severe phenomena of variola. That Jenner (46) recognized the diagnostic significance of this phenomenon, which was exactly analogous to the cutaneous reactions of to-day, is shown by his comments concerning it. The so-called immune reactions did not come into general use as a means of diagnosis until over one hundred years later, when the tuberculin reaction was introduced by Koch and the changes in the blood serum occurring with immunity were studied.

For the purpose of convenience the diagnostic immune reactions may be divided into: (1) reactions which may be carried out in vitro, and (2) reactions which must be observed in the body of the patient.

DIAGNOSTIC REACTIONS IN VITRO

Methods of Obtaining Serum.—Reactions in vitro are concerned for the most part with the serum of patients. The methods of obtaining serum for examination vary according to the amount required. For the microscopic agglutination test a drop of blood is sufficient. The technique of obtaining this is perhaps familiar to all. The lobe of the ear or the finger is thoroughly cleansed with alcohol and then dried with a piece of sterile gauze. A puncture is made with a small lancet such as is devised for obtaining blood for counts. A Hagedorn needle answers the purpose very well. The instrument should be sterilized with alcohol or carbolic acid. A few drops of blood are then collected upon a clean microscopic slide or a piece of heavy tin foil. Each drop should be separate from the others.
This method is particularly adapted to cases where the blood is to be kept for a time or sent to a laboratory. In order to obtain small amounts of serum the same procedure may be used except that the blood is collected in a test tube. By milking the ear amounts of blood up to .5 c. c. may be readily collected. The corpuscles are separated from the serum by centrifugation or by allowing the blood to clot, when the serum will be expressed during the contraction of the fibrin. If centrifugation is used the clot must be separated from the sides of the tube with a clean wire. If the second procedure is used the blood should be slanted in the tube in much the same way as an ordinary agar slant is made. After the clot forms the tube may be returned to the upright position, and the contraction of the clot will be followed by the collection of the serum at the bottom of the slant. For larger amounts of blood the following procedure is used. A syringe of the Luer type with about a No. 18 needle is sterilized by boiling, or, better,

![Fig. 1.—Aspirator for Obtaining Blood.](image)

by dry heat. If the syringe is boiled, care should be taken that all of the water possible is expelled from the syringe, as otherwise some laking of the blood will take place. The skin over the median basilic vein of the arm is carefully cleansed with sterile gauze soaked in alcohol; a fifty per cent. solution is preferable. Enough friction should be used to remove the superficial layers of the epidermis and to produce a hyperemia without trauma to the deeper layers. (It is very easy in some cases to produce considerable hemorrhage.) A constrictor such as a soft rubber tube is then placed around the upper arm in the region of the middle of the biceps in such a way as to cause a congestion of the veins of the arm without occluding the arterial blood supply. The needle, held nearly parallel with the vein, is then inserted through the skin over the median basilic vein, in the direction of the blood stream, and into the lumen of the vein. The tendency of the vein to roll to one side may be prevented by holding the vein firmly in position and putting slight traction on the skin above it by means of the thumb of the hand not engaged in manipulating the syringe. When the point of the needle is within the vein the blood is aspirated into the syringe and the constrictor removed. The needle is then withdrawn and any oozing of blood prevented by holding a sterile gauze sponge firmly over the punctured wound. Elevating the arm will also help to prevent bleeding. The constrictor must always be removed before the needle is withdrawn, otherwise a hematoma of considerable size may form.

The blood is then ejected into a clean test tube or centrifuge tube. If centrifugation is not to be used in separating the serum the tube should be slanted until clotting occurs, to facilitate the expression of serum by the
clot. As a substitute for a syringe, the aspirator shown in the cut has been found very convenient. It consists of a glass tube drawn out at both ends. To one end is fitted a rubber connection, and to this, an ordinary antitoxin needle. To the other end is attached a piece of rubber tubing, through which suction can be made with the mouth. A piece of cotton is placed distal to the constriction in that end of the tube to which the mouth rubber is attached. The instrument is sterilized in an autoclave with the rubber connection in position and covered with cloth or with a small test tube. The needle is sterilized separately and attached at the time of use. At times other veins than those of the arm are more readily accessible. In children the veins of the dorsum of the foot are often most easily entered. In babies the external jugular is often the only accessible vein. It becomes prominent during spasms of crying.

A convenient way of removing the serum from the clot or supernatant fluids in general is by means of a pipette of glass tubing drawn out at one end into a sealed capillary, and plugged at the other end with cotton, which does not project from the lumen of the tube. These tubes of various sizes are sterilized by hot air and kept on hand. When used the end of the capillary is broken off and a rubber tube slipped over the large end of the pipette. Suction is applied to the end of the rubber tube by means of the mouth or a rubber nipple.

The serum reactions depend upon interactions of antibodies and antigens. These reactions may be considered here as of two kinds: (1) Those in which there is a readily discernible change resulting from the interaction of antigen and antibody. These include agglutination and precipitation. (2) Those reactions in which some sort of indicator is necessary to demonstrate the change resulting from the interaction of antigen and antibody. To this class belong the opsonic reaction, complement-fixation reaction, miostagmin reaction, epiphanin reaction, and the ferment reactions of Aberhalden. As the reactions of the first group involve the simpler technique, they will be considered first.

The Agglutination Reaction

Immediately following the description of the phenomenon of agglutination as an immune reaction by Gruber and Durham in 1896, the blood serum of typhoid patients was tested and found to give the reaction, and the test came rapidly into use for the diagnosis of typhoid fever. Widal was the first to report any considerable number of cases, and the reaction has become known, in its application to typhoid, as the Widal reaction. The test depends upon the fact that, consequent upon infection, substances known as agglutinins are found in the blood serum of the infected individual which are capable of clumping together the specific organisms of the infection (agglutinogen). The result is that, when a homogeneous
suspension of organisms is acted upon by their specific agglutinin, they are brought together in groups and fall to the bottom of the fluid in which they are suspended. The homogeneously turbid fluid is thus replaced by a clear one with a sediment which, when shaken, consists of relatively coarse flocculi.

**Specificity of the Reaction.**—The reaction is specific. That is, agglutinins developed by certain organisms will clump only those organisms which produced them, with the following exception. Many organisms contain certain chemical groupings which are found in other organisms as well. These groupings common to two or more organisms give rise to common agglutinins, and the more chemical groups the organisms have in common the more strongly will be developed the common agglutinins. The agglutinins common to two or more organisms are called coagglutinins, and are of course more markedly developed in infections by bacteria which closely resemble one another as do the members of the typhoid and paratyphoid group.

*Agglutination Reaction in Typhoid Fever (Widal Reaction)*

**The Typhoid Culture.**—The typhoid bacilli for the agglutination test may be obtained from the blood of a patient with typhoid or from the spleen at autopsy. The bacilli are short, plump, motile Gram-negative rods, about 0.5 to 0.8 μ in breadth and 1 to 3 μ in length. The typhoid bacillus does not form gas in dextrose media, does not form acid in lactose and saccharose, but does form acid in milk and in mannite. Indol is not formed. These cultural reactions should always be observed in selecting a strain of typhoid bacilli for the agglutination test. Not all strains of typhoid bacilli are adapted to the agglutination test. As a rule, strains which have been grown for some time on artificial media are less likely to show either of the following undesirable qualities: (1) inagglutinability or hypagglutinability, and (2) spontaneous agglutination. Some strains are very readily agglutinated by normal serum, or even show spontaneous agglutination. When a culture is obtained which answers the requirements, it may be grown in broth or on agar slants. Cultures should be used which are at the height of their growth, i. e., about twenty-four hours old. Cultures grown at room temperature are preferred by many to those grown at 37° C. If agar slants are used the organisms are washed off the slant into a few cubic centimeters (1 to 2 drams) of salt solution. If the suspension

* Cole (2) describes a strain with which only slight agglutination could be obtained with highly potent serum. Gay and Claypole (34) state that two transplantations on 10 per cent. rabbit’s blood agar rendered typhoid bacilli nonagglutinable in an immune serum produced with plain agar cultures. This is not borne out by the work of Bull and Pritchett (13).
contains any clumps they may be removed by light centrifugation for a minute or two.

The Microscopic Reaction.—The serum is diluted by placing one drop of the serum into a clean receptacle such as a small test tube or watch glass and adding with the same pipette 9 drops of salt solution or broth. Instead of a dropper a loop of platinum may be used, one loop of serum being added to 9 loops of diluting fluid. One drop or loop of the 1-10 dilution is then added to an equal quantity of diluent, making a 1-20 dilution. In case a drop of dried blood is used, a drop of tap water approximating the size of the drop of blood is added to it and mixed thoroughly with it. A dilution of one part of this mixture with 9 parts of water is then made. Inasmuch as approximately one-half the blood is serum, this makes a 1-20 dilution. The same result may be obtained by a 1-10 dilution of blood made at the bedside with the ordinary white blood corpuscle counting pipette, using plain water as a diluting fluid to take the blood. The 1-10 dilution of blood equals a 1-20 dilution of serum.

The mixture of bacteria and diluted serum is then made by adding a loop of the bacterial suspension to a loop of serum dilution on a cover glass, thus making a final dilution of 1-40. The cover glass is then inverted over the concavity of a hollow-ground glass slide. The edge of the cover is sealed with vaselin and the hanging drop observed with the No. 7 objective. Although it is preferable, the hanging drop is not necessary, and the reaction can be observed, using an ordinary slide and cover slip. A control may be made by the same procedure, using broth or salt solution instead of serum dilution. As the positive reaction is observed, it will be seen that the actively motile organisms become less active and begin to form small groups, which tend constantly to grow larger, while the intervening spaces become free from bacteria. Care should be taken not to confuse masses of erythrocyte shadows with bacteria where whole blood is used.

The reaction usually begins within one-half hour, and is strongly agglutinating serums may take place almost immediately. In case of the negative reaction in the control the organisms continue to move about the field in more or less even distribution. The reaction is to be considered negative if clumping does not occur in one hour. If it is desirable to ascertain the degree of dilution with which clumping will occur, serial dilutions can be made by adding equal parts of diluent to the progressive dilutions. Slides with multiple concavities are made so that a number of dilutions can be observed at once.

The Macroscopic Method.—The gross test is best carried out in small test tubes which will easily hold about 2 c. c. (½ dram). A good size is about 7 cm. (3 in.) long with a lumen about 7 mm. (½ in.) in diameter. Many prefer tubes drawn out to a cone at the bottom to facilitate observation of the sediment. Great care should be used in cleaning the tubes and
freeing them from all cleaning reagents. Dilutions can be made by the drop method or more accurately by means of a 1 c. c. pipette marked with .01 c. c. graduations. If the drop method is used the fluid is dropped from tubes drawn out into capillaries as described for removing serum from clots. The same tube should be used for all fluids concerned in the test, as the size of the drops will vary according to the caliber of the capillary. The dropper should always be held at the same angle, as this, too, influences the size of the drops. A series of mixtures by means of drops may be made by arranging a row of tubes in a small tube rack and putting the serum, diluting salt solution, and bacterial suspension into them according to the following table. The suspension should be of such a strength as to render the final mixture distinctly opalescent (about 2 c. c. to an agar slant).

### Arrangement of Dilutions for Gross Agglutination Test

<table>
<thead>
<tr>
<th>Tube</th>
<th>Serum</th>
<th>Salt solution</th>
<th>Bacterial Suspension</th>
<th>Final Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4 drops</td>
<td>15 drops</td>
<td>1 drop</td>
<td>1-5</td>
</tr>
<tr>
<td>2</td>
<td>2 drops</td>
<td>17 drops</td>
<td>1 drop</td>
<td>1-10</td>
</tr>
<tr>
<td>3</td>
<td>1 drop</td>
<td>18 drops</td>
<td>1 drop</td>
<td>1-20</td>
</tr>
<tr>
<td></td>
<td>Serum dilution</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1-1 1 drop</td>
<td>18 drops</td>
<td>1 drop</td>
<td>1-40</td>
</tr>
<tr>
<td>5</td>
<td>1-4 1 drop</td>
<td>18 drops</td>
<td>1 drop</td>
<td>1-80</td>
</tr>
<tr>
<td>6</td>
<td>1-8 1 drop</td>
<td>18 drops</td>
<td>1 drop</td>
<td>1-160</td>
</tr>
<tr>
<td>7</td>
<td>1-16 1 drop</td>
<td>18 drops</td>
<td>1 drop</td>
<td>1-320</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>19 drops</td>
<td>1 drop</td>
<td></td>
</tr>
</tbody>
</table>

In the centimeter method the dilutions are carried out as follows: A series of tubes is arranged as before, and in the first tube a dilution of 1-10 is made by adding .1 c. c. of serum to .9 c. c. of salt solution. Into each of the succeeding tubes is put .5 c. c. of salt solution. Then .5 c. c. of the dilution in tube one is added to the .5 c. c. of salt solution in tube two, thus doubling the dilution. Then .5 c. c. of number two is added to tube three, etc., until the last tube, when .5 c. c. is discarded. Then to the fluid (.5 c. c.) in each tube is added .5 c. c. of a bacterial suspension, which should be of a strength of about 1 agar slant to 10 c. c. of salt solution. A control is made of equal parts of suspension and salt solution. After the mixtures are made up they are shaken and incubated at 37° C. for two hours, then examined. In a positive test clumping can be readily seen. The fluid is clear with the bacteria in large flocculi at the bottom of the tube. If now the tubes are allowed to stand in the ice box over night and examined in 12 to 24 hours, the positive tubes will be found covered at the bottom with a film which extends up to the sides of the tube, while in the negative tubes the sediment will be in the form of a button at the bot-
tom of the concavity. Owing to the fact that the gross test especially can be successfully carried out with killed cultures of typhoid which will keep indefinitely, various outfits are put on the market.

**Comparison of the Gross and Microscopic Tests.**—There has been considerable discussion as to which test is more reliable. The use of the one or the other should be decided according to the use to which they are to be applied. Either test is reliable, but for cases where a single dilution is to be tried or a small amount of serum is available the microscopic test is preferable. Where a large series of dilutions is to be made the gross test is much more convenient.

**Diagnostic Value of the Agglutination Test in Typhoid.**—*Sources of Error.*—Figures collected by Paltauf (69) show that the percentage of cases of typhoid fever giving a positive Widal test varies from 73½ per cent. to 99½ per cent. The figures, however, average about 95 per cent. Cases in the first week of the disease, according to Paltauf's figures, give a positive Widal in 75 per cent., in the second week 90 per cent., and in the third week 95 per cent. The reaction, however, rarely appears before the latter part of the first week. Wendel (88) reports cases in which the agglutinating property of the serum appeared late in convalescence. Jochmann (48) reports five cases with positive bacteriological examination in which there was throughout the disease a fully negative Widal. On the other hand, it is not to be forgotten that many cases show a persistent positive test many years after typhoid. Thus Iversen (42) reports an agglutination of 1-100 as long as 10 years after an attack of typhoid. Typhoid vaccination also causes a persistent Widal reaction.

[A positive agglutination reaction occurring in persons who years before have suffered from typhoid suggests the possibility that these individuals still harbor typhoid bacilli, i. e., are typhoid carriers. Thus, agglutination tests have been suggested and employed for the detection of carriers responsible for the infection of milk and other food supplies which have given rise to outbreaks of typhoid fever. After antityphoid vaccination the agglutination may fall to normal within a few weeks or months, while the immunity to typhoid persists for a longer period.—Editors.]

Other conditions giving a positive Widal collected by Kreissl (54) are cirrhosis of the liver, carcinoma of the stomach, ovarian cyst, adiposity, phosphorus intoxication, and icterus. It must be said, however, that in such cases a previous infection is possible and that confusion in diagnosis from such reaction is rare. In mixed infections, according to Kayser (49), there may be a negative Widal. Influenza, malaria, miliary tuberculosis, and pneumonia are mentioned. Rarely a negative reaction may be caused by inhibition in lower dilutions where a strongly agglutinating serum is present. (Ceritto, quoted by Kreissl.) By far the most important cause
of confusion is the presence of coagglutinins discussed on page 120. The serum from any of the paratyphoid group of infections may agglutinate typhoid bacilli in high dilution. In this case two methods are of value in differentiation. First: in most cases the serum of a given infection of this group will agglutinate the specific organism of that infection in higher dilution than it does the other organisms of the group. This, however, is not always the case. Thus Svenstad (69) reports a case of infection by Gaertner's bacillus in which the bacillus enteridis was agglutinated in a dilution of 1-50; bacillus typhosus in a dilution of 1-100. Second: absorption experiments (Castellani's experiment). This procedure makes use of the idea that a certain organism 1 stimulates the formation of two agglutinins which we will call A and B, and a second organism 2 stimulates the formation of agglutinins B and C. Then B will be the common agglutinin and A and C will differ. Now, if a serum containing A and B agglutinins is treated by the homologous organism 1 in excess, all of the agglutinins A and B will be absorbed, leaving no agglutinin for organism 2. If, however, the same serum containing A and B agglutinins is treated with organism 2, only agglutinin B will be absorbed, leaving still an agglutinin for organism 1. Thus in a case of paratyphoid infection giving a positive agglutination test with typhoid organisms absorption of agglutinin with the paratyphoid organism will result in no remaining agglutinin for typhoid bacilli. Absorption of the same serum with typhoid bacilli, however, will always leave some agglutinin for paratyphoid.

Agglutination Reaction in the Paratyphoid Infections

In the paratyphoid infections the agglutination tests are carried out in the same way as in typhoid. The reaction has also about the same value. In paratyphoid A coagglutinins for typhoid are more common than in paratyphoid B infections.

Agglutination Reaction in Dysentery

The agglutination reaction is more unsatisfactory in the diagnosis of dysentery, according to most observers, on account of tardy development or complete failure of agglutinin formation. Lucas, Fitzgerald, and Schorer (58) give the following figures: Infections with bacilli of the Flexner type gave positive reactions in 57 per cent. of cases; infections with bacilli of the Shiga type in 26 per cent. of cases. Shiga infections are more likely to give coagglutinins for the Flexner type than vice versa. A positive reaction is, however, of diagnostic value.

Agglutination Reaction in Malta Fever

Wright in 1897 first made use of agglutination in the diagnosis of malta fever, and it has since been extensively utilized in diagnosis. Wright
found that the degree of dilution at which agglutination occurred ranged from 1-100 to 1-1000. Coagglutinins for typhoid have been reported in a case by Currey (22). Basset (7) recommends a dilution of 1-30 for diagnosis.

**Agglutination Reaction in Plague**

The agglutination test in plague usually appears too late to be of great value in diagnosis, appearing first between the ninth day and the time of convalescence. A positive reaction, however, is of value, as it is specific and not found in other diseases. The reaction is of most value in the diagnosis of convalescents for the purposes of preventing the spread of the disease.

**Agglutination Reaction in Other Diseases**

Agglutinins for the specific organisms of many other infections have been observed in the serum of patients, but owing to the inconstancy and the many sources of error such as spontaneous agglutination and normal agglutinins the reactions are of but little diagnostic value. By artificial immunization of animals, however, sera of high agglutinating powers may be produced which are of value in differentiating the organisms found in connection with these diseases.

**Precipitin Reactions**

When filtrates from bacteria causing certain diseases are mixed with immune serum obtained from persons suffering from the diseases, precipitates occur in the mixtures. The substances in the immune serum producing this precipitation are known as precipitins. Many attempts have been made to utilize this reaction in diagnosis, but on account of the greater ease with which diagnosis can be accomplished by other means the reaction has not been of much service. Vincent and Bellot (83) have devised a diagnostic test for epidemic meningitis which is carried out as follows: To from 3 to 6 c. c. (1 to 2 drams) of cerebrospinal fluid freed from opacity by centrifugation is added one drop of anti-meningococcic serum. The mixture is allowed to stand 8 to 12 hours at 50° C. to 53° C. and then observed. In positive cases a precipitate occurs. The authors state that the test occurs in cases where meningococci are not obtained either in smears or cultures. These results find confirmation in the work of others. It will be seen that the test depends upon the presence of the antigen in the cerebrospinal fluid instead of the antibody or precipitin which is supplied by the artificially produced serum.

**Complement-Fixation Reaction**

In the agglutination and precipitin reactions we have had examples of serum reactions in which the result of the interaction of antigen and anti-
body was directly discernible. In the same way the result of the action of lysins is readily manifested by the solution or precipitation of the antigenic substances by the antiserum. It occurred to Bordet and Gengou (10) that, in cases where there was no visible result of the antibody-antigen reaction such as solution, the proof of such interaction might still be possible by indirect means. The immune lysins are bodies composed of two portions, one a heat sensitive or thermolabile part (complement) made inactive by heating to 56° C. for one-half hour, and a heat resistant or thermostable part which is called amboceptor. The amboceptor or immune body is the specific part of the lysin, and connects the complement and amboceptor. In the presence of antigen and amboceptor specific for each other complement is bound or fixed to the antigen by the amboceptor. Bordet's idea was that, in the case of insoluble antigens, immune bodies existed which would fix or bind complement to the antigen without necessarily causing the solution or any other visible effect upon the antigen. These bodies he called complement-fixation antibodies, and devised the following method of proving their presence.

In order for a solution of red blood cells to take place by means of their specific immune lysin both the complement and amboceptor must be present and free to act. If, therefore, to a mixture into which we have put complement we add after a time red blood cells and their specific amboceptor, the presence of unfixed active complement will result in hemolysis, a solution of red blood cells, whereas the fixation of the complement will be manifested by a failure of hemolysis.

Bordet and Gengou made use of the phenomenon of complement-fixation in testing for the presence of antibodies to typhoid bacilli in the following way: A suspension of typhoid bacilli was used as antigen and mixed with complement and the serum to be tested. After incubating, to allow the complement to become fixed to the antigen (typhoid bacilli), a quantity of red blood cells and enough of their specific amboceptor to cause hemolysis with the amount of complement in the incubated mixture were added. Now, if the typhoid antibodies were present there was an absence of hemolysis, because the complement had been fixed by means of the typhoid antibodies to the typhoid antigen. In case there were no typhoid antibodies, there was no fixation of complement and hemolysis of the red cells occurred. It is thus seen that the hemolytic system acts simply as an indicator to demonstrate an action otherwise without manifestation.

It was shown by Wassermann and Bruck (86) that extracts of bacteria also might serve as antigen. The reaction has since developed into one of the most valuable means of biological diagnosis. The specificity and delicacy of the reaction make possible the demonstration of such minute quantities of antigen as exist in the circulating blood of acute infections, or, on the other hand, the presence of antibodies may be demonstrated in this way by means of a known antigen.
Complement-Fixation in Syphilis (Wassermann Reaction)

In 1906 Wassermann, Neisser, and Bruck (87) made use of the Bordet-Gengou phenomenon in the diagnosis of syphilis as follows: They were at that time unable to obtain pure cultures of spirocheta pallida for an antigen. They therefore made an extract of the liver of a syphilitic fetus which had been observed to contain great quantities of spirochetes. With a similar idea, Detre (23), a few weeks later, published a method of diagnosis, using an antigen obtained from condylomata. Wassermann, Neisser, and Bruck found that by mixing their antigen with syphilitic serum inactivated by heating to 56° C. and with complement they could obtain fixation of the complement, as evidenced by the fact that, if sheep's erythrocytes and their specific amboceptor were added later, no hemolysis of the corpuscles occurred. The serum of non-syphilitic individuals did not give the reaction. The modifications of this technique, which will be described, have proved to be of great value in the diagnosis of syphilis.

Technique of Wassermann Test.—Apparatus.—The following apparatus is desirable for making the test. A number of 1 c. c. pipettes marked to the tip with .01 c. c. graduations, a few 10 c. c. pipettes graduated to .1 c. c. Test tubes about 8 to 10 mm. in diameter on the inside and about 8 to 10 cm. long. Some graduated cylinders for making corpuscle suspensions. A quantity of sterile (boiled) salt solution (sodium chlorid, 9 grams; water, 1 liter) and test tube racks made with flat tops and parallel rows of holes to fit the small tubes mentioned. Capillary pipettes such as were mentioned in the description of the handling of serum may be used instead of graduated pipettes, and drops instead of measured quantities. The latter method is neither as easy nor as accurate as that with the graduated pipettes. The glass receptacles should be thoroughly cleansed, rinsed, and sterilized by dry air at from 150° to 200° C.

Reagents.—The various reagents taking part in the reaction, it will be remembered, are: the patient's serum, antigen, complement, erythrocytes, and their specific amboceptor. The different ways of preparing these will first be considered and then the ways of combining them.

Patient's Serum.—The technique of obtaining the serum has already been described. Craig (21) has called attention to the necessity of maintaining the sterility of the serum to be tested, as the action of bacteria on the serum influences the outcome of the test. It is well to provide about 2 c. c. of serum in order to have enough for the test and more if a repetition is required. The serum may be inactivated, that is, made free from active complement, by heating to 55° C. for one-half hour in a water bath or may be used without heating, according to the method employed. In the original reaction as performed by Wassermann, as has been stated, the serum was heated to 56° C. for one-half hour in order to destroy the native complement and thus have only the constant quantity which was added.
The work of Noguchi (67) has shown that the complement may be neglected, but that there exists instead of this reason a more important one for heating the serum to be tested. The syphilitic complement-binding body begins to be destroyed, according to Noguchi, at 45° C., and is completely destroyed at 75° to 80° C. Heating to 55° C. affects it, however, but little. Certain other substances sometimes occur in normal or non-syphilitic serums which are capable of causing fixation of complement when combined with autolytic protein decomposition products which occur in some preparations of antigens. Noguchi has termed such fixation "proteotropic complement-fixation," and is able to avoid such fixation by acetone insoluble antigens, which are free from protein decomposition products such as albumoses and peptones.

It will be seen, therefore, that, when acetone insoluble antigen is used, unheated serum may also be used. With other preparations of antigens heated serum should be used. On account of the partial destruction of the syphilitic complement-fixation body, the test with heated serum is not so delicate as when unheated serum is used. It is desirable, therefore, to use twice as much heated serum as unheated serum. Serums that have stood for some time should always be heated as anti-complementary substances develop, which are destroyed by heating at 55° C. for 20 minutes. Serums which have been taken after meals have in them similar substances, and it is desirable to take the blood for the Wassermann test before meals.

There exists at times in serums a natural amboceptor for such erythrocytes as sheep's, beef's, etc. There are two ways of dealing with these serums. The first is to add erythrocytes to the serum and then remove them when the amboceptor will be absorbed and removed with the cells, which are separated by simple centrifugation. The second procedure is to estimate the natural amboceptor and use it in place of an artificial one.

Antigen.—The original Wassermann was done with a watery extract of syphilitic liver. This antigen was unsatisfactory in that it changed very easily in strength. Alcoholic extracts were therefore tried and found to be satisfactory substitutes. It was then shown by Landsteiner, Mueller, and Poetzl (55) that alcoholic extracts of non-syphilitic liver could be used with even better results than those obtained from syphilitic organs. They showed, furthermore, that alcoholic extracts of heart muscle made a very satisfactory antigen. With the purpose of producing an antigen which would keep well and give the highest possible percentage of positive reactions in syphilis without being so sensitive as to give positive tests with normal serum, a great number of preparations have been tried. Of these preparations two have been found especially valuable.

1. Acetone Insoluble Extract of Heart.—Noguchi (67) gives the following method of preparation: Minced beef heart is mixed with ten times its volume of 95 per cent. alcohol and extracted for one week at 37° C. The extract is then filtered through filter paper and evaporated by means
of a fan at a temperature of about 37° C. or less. Enough ether is added to the residue to dissolve it, and then 10 volumes of acetone are added. The supernatant acetone is decanted and the mass evaporated before a fan. The resinous substance is then preserved in an air-tight vessel of glass. The preservation of this antigen in a 3 per cent. solution in methyl alcohol has been found more satisfactory than keeping it in suspension in salt solution. For making the test a 1 per cent. suspension of the methyl alcohol solution in 0.9 per cent. sodium chloride solution is in this way a fresh suspension, and is readily prepared for each test.

2. Cholesterinized antigens, advocated by Sachs (74), McIntosh and Fildes (61) and Walker and Swift (84). Walker and Swift recommend the following procedure for the preparation of cholesterinized alcoholic extract of the human heart. "The tissue free from fat was minced, weighed and placed in absolute alcohol in the proportion of one gram of tissue to 10 c. c. of alcohol. Extraction was carried on in the incubator at 37° C. for two to three weeks. The bottle was shaken daily. The extract was cooled to room temperature and filtered. The filtrate was used as a stock solution. To this stock solution 0.4 per cent. of cholesterin was added."

We have found it well to use both these antigens as a routine. Most observers have found the cholesterinized alcoholic extract to be more sensitive but to give a higher per cent. of positive reactions in cases in which the probability of syphilis is slight.

Complement.—For complement normal guinea-pig serum has been found most satisfactory, although many tests have been devised in which the complement present in the serum to be tested is used (Tschernogobow, 80). The guinea-pig serum is obtained by aspiration of blood through the needle of a Luer syringe or other aspirator inserted into the animal's heart. Animals may be bled in this way a number of times if only small quantities (3 to 5 c. c.) are required. The serum may also be obtained by cutting the guinea-pig's throat and collecting the blood in a test tube. The serum may be obtained by centrifuging or allowing the blood to clot in a slant in the tube. Serum over 24 hours old should not be used.

Erythrocytes.—Although other systems are in use, the most common ones are those of the human erythrocytes recommended by Noguchi (67) and sheep erythrocytes recommended by Wassermann (86). Human blood may be conveniently obtained by taking a little more than required for the test from the patient to be examined. One c. c. of blood is added to 9 c. c. of a 1 per cent. sodium citrate in .9 per cent. sodium chloride solution. The blood and citrate solution are then centrifugated until the supernatant fluid is free from corpuscles and the fluid decanted. This process is repeated twice each time, filling the centrifuge tube with normal salt solution. After decanting the salt solution the corpuscles now free from serum are suspended in 9 c. c. of normal salt solution, making a 10 per cent. suspension of red cells. Sheep's blood may be obtained from slaughtered animals, in
which case the blood is caught in a clean, dry bottle and defibrinated by shaking with glass beads or beating with a clean wire. A definite measured quantity of the defibrinated blood is then washed in salt solution twice, as above, and suspended in 19 volumes of salt solution, thus making a 5 per cent. suspension of red cells. The blood may also be obtained from the sheep by aspiration through a needle inserted into the jugular vein.

Amboceptor.—The most satisfactory amboceptor is the heated serum of rabbits immunized to erythrocytes. The sheep’s blood is obtained as described in the preparation of erythrocytes and defibrinated. The blood corpuscles are then washed three times in salt solution as described and made up into the same volume as the blood before washing. The rabbit is injected intraperitoneally every five days with 3 c. c. of blood the first injection, 6 c. c. the second injection, 10 c. c. the third, 15 c. c. the fourth, and 20 c. c. the last injection. About 10 days after the last injection the animal is bled by cutting one of the marginal veins of the ear with a sharp knife. The amboceptor thus obtained is titrated (see below), and if sufficiently strong the animal is bled by dissecting out the carotid artery and inserting a cannula or by aspiration through a needle thrust into the heart. The blood is collected in a sterile glass cylinder and slanted until clotted. It is then placed in the ice box until the serum separates, when it is removed from the clot by means of a pipette and inactivated by heating to 56° C. for one-half hour. The serum may be sealed in small tubes and kept in a dark, cool place indefinitely.

Choice of Method.—There are now a great many different modifications of the Wassermann test, a number of which are undoubtedly efficient. The ones which will be found most satisfactory are: (1) the Noguchi modification of the Wassermann test, in which acetone insoluble antigen is used with a sheep-anti-sheep system as indicator, and (2) the Noguchi reaction with human cells. The choice between these two methods will depend more on convenience than on efficiency, as they are both satisfactory if properly controlled. The Noguchi human system avoids the chance of error, due to anti-sheep amboceptor in the test serum, by using human corpuscles. The same may be done by proper controls, using the sheep-anti-sheep system. It is easier to produce a highly potent anti-sheep rabbit serum than to produce a highly potent anti-human rabbit serum. Then, again, the human red cells for the suspension to be used are more easily obtained than are those of the sheep. A way of carrying out these methods which has proved very satisfactory is similar to that described by MacRae, Eisenbrey, and Swift (60).

Standardization of Reagents.—Amboceptor Titration.—The amount of amboceptor necessary for the test is determined as follows: The procedure is the same for both human and sheep system, excepting that in the human system .1 c. c. of 10 per cent. red cells is used and in the sheep system .5 c. c. of 5 per cent. red cells is used. Graded amounts of ambo-
Contents of Tubes for a nboceptor Titration

<table>
<thead>
<tr>
<th>Complement 10% e. c.</th>
<th>Ambroceptor 1% e. c.</th>
<th>Sheep Cells 5% e. c.</th>
<th>Salt solution to e. c.</th>
<th>Result Hemolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.025</td>
<td>0.5</td>
<td>2.5</td>
<td>None</td>
</tr>
<tr>
<td>0.5</td>
<td>0.05</td>
<td>0.5</td>
<td>2.5</td>
<td>Trace</td>
</tr>
<tr>
<td>0.5</td>
<td>0.1</td>
<td>0.5</td>
<td>2.5</td>
<td>Moderate</td>
</tr>
<tr>
<td>0.5</td>
<td>0.2</td>
<td>0.5</td>
<td>2.5</td>
<td>Complete</td>
</tr>
<tr>
<td>0.5</td>
<td>0.3</td>
<td>0.5</td>
<td>2.5</td>
<td>Complete</td>
</tr>
<tr>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>2.5</td>
<td>Complete</td>
</tr>
<tr>
<td>0.5</td>
<td>1.0</td>
<td>0.5</td>
<td>2.5</td>
<td>Complete</td>
</tr>
</tbody>
</table>

Complement and red cells are put into a series of tubes with constant quantities of complement and red cells and the tubes incubated for one hour. An observation is then made to find out the smallest quantity of amboceptor which has completely dissolved the red cells. In case hemolysis is complete the opaque suspension becomes a clear red solution. The lowest amount of amboceptor necessary for complete hemolysis is called one unit of amboceptor. The protocol shown above illustrates such a titration. In this case .2 c. c. of 1 per cent. amboceptor constitutes 1 unit.

**Determination of the Syphilitic Unit.**—In order to standardize a new antigen it is necessary to have a standard syphilitic serum with which to work. This is found as follows: To a series of tubes containing constant quantities of standard antigen and complement varying quantities of a known syphilitic serum are added. These mixtures made up to a constant quantity are incubated for one hour, and then 2 units of amboceptor and .5 c. c. of erythrocyte suspension are added, and the mixture incubated again. The smallest quantity of serum which will fix the complement is called the syphilitic unit. Such a protocol is as follows:

**Contents of Tubes for Determination of Syphilitic Unit**

<table>
<thead>
<tr>
<th>Amount Known Positive Serum e. c.</th>
<th>Complement 10% e. c.</th>
<th>Standard Antigen Emulsion</th>
<th>Procedure</th>
<th>Result Hemolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.5</td>
<td>0.5</td>
<td>Total volume made to 1.5 c.c. and incubated 1 hour. Then .5 c.c. erythrocytes and 2 units of amboceptor made to .5 c.c. added and incubated 1 hour.</td>
<td>None</td>
</tr>
<tr>
<td>0.05</td>
<td>0.5</td>
<td>0.5</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>0.025</td>
<td>0.5</td>
<td>0.5</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>0.012</td>
<td>0.5</td>
<td>0.5</td>
<td>Slight</td>
<td></td>
</tr>
<tr>
<td>0.006</td>
<td>0.5</td>
<td>0.5</td>
<td>Moderate</td>
<td></td>
</tr>
</tbody>
</table>

In this case 0.025 c. c. of serum equals the syphilitic unit.

**Determination of the New Antigen Unit.**—Using now the unit of syphilitic serum in constant quantity, varying amounts of new antigen emulsion are used in order to find the lowest quantity necessary to fix complement. This quantity is called the unit of antigen. The following protocol will illustrate:
Two controls are made. One as above, excepting that normal serum is substituted for syphilitic. In this set of controls no fixation of complement should occur in at least 20 times the quantity determined as the antigen unit. A second control is made with antigen and corpuscle suspension alone to see if the extract alone is hemolytic for blood cells. If hemolytic or capable of fixing complement alone or with normal serum the antigen must be discarded as unsatisfactory. The quantities of each of the reagents necessary to use having been determined, the test of the suspected serum is carried out as follows:

**Wassermann System with Heated Serum**

A series of seven test tubes is arranged in a rack, and into each is measured 0.5 c. c. of complement. Into No. 1 are put 0.1 c. c. of serum to be tested and 4 units of antigen. Tube 2 is made up as tube 1, except that the antigen is omitted. Tube 3 differs from tube 1 in that 0.1 c. c. of known positive serum is used instead of the test serum. Tube 4 is as 3, but without antigen. Tube 5 contains the same reagents as 3, excepting that either 0.1 c. c. of salt solution or 0.1 c. c. of normal serum is added, instead of positive serum. Tube 6 is as tube 5, excepting that it is without antigen. Into tube 7 are put 0.5 c. c. of heated suspected serum and 0.5 c. c. of sheep’s corpuscle suspension. Tube 8 is the same as 7, excepting that it has 0.1 c. c. of suspected serum instead of 0.5 c. c. Tube 9 may be used for a second antigen (see page 129). The tubes are now all made up to 1.5 c. c. and incubated for 1 hour at 37° C. At the end of that time 0.5 c. c. of corpuscle suspension and 2 units of amoceptor made up to 0.5 c. c. are added. In the event that complete hemolysis has occurred in tube 7 the amoceptor is omitted from 1 and 2. If complete hemolysis has not taken place in 7 but has in 8 one unit of amoceptor is added to 1 and 2. The tubes are now incubated for an
hour and again observed. In case the test is positive the opaque corpuscle suspension will be unaffected. If it is negative there will be hemolysis. The controls should be as follows: Tubes 2, 4, and 6 should be hemolyzed, as there should be no fixation of complement without antigen. Tube 3 should show no hemolysis, as there should be fixation of complement with the syphilitic serum. Tube 5 should be hemolyzed as the antigen alone, or with negative serum, should not fix complement.

The Noguchi system with heated serum is carried out the same as in the above, except that in the second step 1 c. c. of a 10 per cent. suspension of human cells is added instead of sheep's cells and 2 units of anti-human amboceptor.

Unheated serum may be used in either test in one-half the quantity of the heated serum, as it is more sensitive.

Cerebrospinal fluid is used in double the quantity of heated serum and is not heated. Hauptmann and Hossli (39) use graded amounts of spinal fluid up to a maximum of 1 c. c. in a total volume of 3 c. c. McIntosh and Fieldes consider it very doubtful if quantities over 0.4 c. c. in 2 c. c. are of value.

In some cases it may be desirable to ascertain how strongly or weakly positive a serum is. This is done as shown in the estimation of the syphilitic unit.

Nature of the Wassermann Reaction.—It will be remembered that the original Wassermann test was based upon the idea of a complement-fixation which was specific in the biological sense of the term. That is, that the complement-fixation body of syphilitic serum and the antigen of the test were analogous to antigen and antibody in typhoid fever or other diseases where pure cultures of antigen were obtainable. The substitution of pure lipoids for extracts of syphilitic organs immediately threw serious doubt upon this view. Since the culture of spirochaeta pallida in pure culture Noguchi (55) has been able to prove that the Wassermann test does not depend upon antibodies as do other complement-fixation reactions, but that it is a special reaction having for its antigen a substance entirely separate from that occurring in the specific organism of the disease. This he has done by using antigen prepared from pure cultures of spirochaeta pallida. In this way he obtains a reaction differing markedly from the Wassermann test.

Complement-Fixation with Spirochetal Antigen.—Animals were immunized by injection of killed cultures of spirochaeta pallida, and in this way an immune serum was obtained. This immune serum gave a complement-fixation with the antigen made from pure cultures of spirochetes, but none with the lipoid antigen of the Wassermann test. Serum from animals with experimental syphilitic orchitis, on the other hand, gave positive reactions with lipoid antigen, but not with that made from spirochetes. It was found, too, that the reaction with antigen of spirochetes
became more marked in patients who had recovered from syphilis, in this way being analogous to other immune complement-fixation reactions such as typhoid. In contrast to this the Wassermann test becomes less marked or negative as the patient recovers.

**Clinical Value of the Wassermann Test.**—**Primary Syphilis.**—The earliest appearance of the reaction, according to Pearce (70), is that reported by Lesser in a case showing a positive reaction 8 days after exposure and 14 days before the initial lesion appeared. This, however, is an exceptional case. Swift (78) found all cases examined positive at the end of the fourth week following the appearance of the chancre.

Noguchi gives the results with his modification from a collection of figures as varying from 72 per cent. (including very early cases) to 100 per cent. with an average of 87.5 per cent. positive. These percentages are somewhat higher than those representing the results with varying methods. Such figures vary from 64 per cent. to 92 per cent., with an average of 78 per cent. of positive tests.

**Secondary Syphilis.**—In secondary syphilis the reaction gives the highest percentage of positives. Noguchi's figures show 96 per cent. positives. Other collections vary from 71 per cent. (an unusually low figure) to 100 per cent., with an average of 85 per cent. positive.

**Tertiary Syphilis.**—In tertiary syphilis the percentage of positive reactions drops again below that of the primary stage. Noguchi's figures are 83 per cent., with other figures varying from 63 to 89 per cent., averaging 76 per cent. positive.

**Latent Syphilis.**—Pearce gives figures from 51 per cent. to 76 per cent. in early latent cases and from 46 to 79 per cent. in late latent cases. The average is about 63 per cent. in both early and late cases.

**Hereditary Syphilis.**—According to Noguchi, hereditary syphilis gives positive reaction in 96 per cent. of cases.

**Cerebrospinal Syphilis.**—Tables by Noguchi average 90 per cent.

**General Paresis.**—The figures of Noguchi are from 80 per cent. to 100 per cent. of positive reactions with the blood serum and from 73 to 100 per cent. with the cerebrospinal fluid.

**Tubes.**—With the blood serum from 40 per cent. to 80 per cent. positive and with the cerebrospinal fluid 50 per cent. to 66 per cent.

The table opposite shows the value of the reaction in different stages.

**Positive Wassermann Tests in Diseases Other Than Syphilis.**—Various authors have reported cases of frambezi (yaws), trypanosomiasis, scarlet fever, leprosy, tuberculosis, carcinoma, jaundice, and diabetes in which positive reactions were obtained. In yaws we have a disease which is caused by an organism very similar to that of syphilis, and it is not surprising that a similar reaction should be found. The absence of both yaws and trypanosomiasis in this country makes confusion in diagnosis unlikely. It has been shown (28) that the positive reactions obtained in scarlet fever
DIAGNOSTIC REACTIONS IN VITRO

Table Showing Value of Wassermann Test in Different Stages of Syphilis

<table>
<thead>
<tr>
<th>Stage</th>
<th>Per Cent. High</th>
<th>Per Cent. Low</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary syphilis</td>
<td>100</td>
<td>72</td>
<td>87.5</td>
</tr>
<tr>
<td>Secondary syphilis</td>
<td>100</td>
<td>92</td>
<td>96</td>
</tr>
<tr>
<td>Tertiary syphilis</td>
<td>89</td>
<td>63</td>
<td>76</td>
</tr>
<tr>
<td>Early latent syphilis</td>
<td>78</td>
<td>51</td>
<td>64</td>
</tr>
<tr>
<td>Late latent syphilis</td>
<td>79</td>
<td>46</td>
<td>63</td>
</tr>
<tr>
<td>Hereditary syphilis</td>
<td>100</td>
<td>92</td>
<td>96</td>
</tr>
<tr>
<td>Cerebrospinal syphilis</td>
<td>89</td>
<td>63</td>
<td>78</td>
</tr>
<tr>
<td>General paresis spinal fluid</td>
<td>100</td>
<td>80</td>
<td>90</td>
</tr>
<tr>
<td>General paresis blood serum</td>
<td>80</td>
<td>66</td>
<td>72</td>
</tr>
<tr>
<td>T. paresis spinal fluid</td>
<td>66</td>
<td>50</td>
<td>58</td>
</tr>
<tr>
<td>T. paresis blood serum</td>
<td>80</td>
<td>66</td>
<td>72</td>
</tr>
</tbody>
</table>

were at least in part due to the use of poor antigens and that a positive reaction occurring in scarlet fever is suggestive of syphilis. The positive reactions in carcinoma, tuberculosis, and diabetes have been connected with a cachectic condition, in which differential diagnosis is easily made by other means.

Influence of Treatment on the Wassermann Test.—The nature of the Wassermann reaction has been discussed. Citron (17) gives his reasons for believing that the positive Wassermann test is a sign of active syphilis:

(1) The constancy of the reaction in manifest syphilis.
(2) The fact that, in untreated or poorly treated cases, it persists for years, whereas the ordinary antibodies usually last a matter of months after the disease has run its course.
(3) The fact that in latent cases treated specifically the Wassermann tends to disappear. The view that the Wassermann test means active syphilis or at least the presence of living spirochetes has become general.

Effect of Treatment on the Wassermann Test

<table>
<thead>
<tr>
<th>Time Treated</th>
<th>Number of Cases</th>
<th>Positive</th>
<th>Negative</th>
<th>Time Treated</th>
<th>Number of Cases</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 weeks</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>40 weeks</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>4 weeks</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1 year</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>6 weeks</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>14 months</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>8 weeks</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>15 months</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>10 weeks</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>18 months</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>12 weeks</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>2 years</td>
<td>6</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>16 weeks</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>30 months</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>24 weeks</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>3 years</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>28 weeks</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>8 years</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>32 weeks</td>
<td>5</td>
<td>1</td>
<td>3</td>
<td>12 years</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>36 weeks</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mercurial Treatment.—The effect of mercurial treatment is seen in the table of Craig (19). Of 52 cases, 32 were positive and 20 negative.

Jenonek and Meirowski (47) give the following results of treatment:
Swift (8) obtained positive reactions in 74 per cent. of cases treated six months or less. This percentage gradually diminished to 37 per cent. in cases treated 3 years. Blaschko found a gradual disappearance of the reaction in 76 out of 90 cases. Regarding the stage of the disease, Swift (8) finds that strongly positive reactions in the tertiary periods are harder to influence than in the first stages, while the maximum benefit is obtained in the primary stage. According to Merz (64), patients treated in the primary stages gave half as many positives as those treated in the later stages. The importance then of early treatment is obvious. It has been a common experience that the secondary symptoms are less likely to appear in cases treated in the primary stage of the disease. The influence of the stage of the disease upon the result of treatment is shown in the table by Swift (78) see below.

### Influence of Treatment on the Wassermann Test

<table>
<thead>
<tr>
<th>Author</th>
<th>Stage</th>
<th>Number of Cases</th>
<th>Became Negative</th>
<th>Weaker</th>
<th>Unaltered</th>
<th>Stronger</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mueller</td>
<td>I</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Fischer</td>
<td>I</td>
<td>17</td>
<td>9</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Reinhart</td>
<td>I</td>
<td>56 negative</td>
<td>45 remained</td>
<td>15</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II early</td>
<td>24</td>
<td>1</td>
<td>9</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II early</td>
<td>13</td>
<td>9</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II early</td>
<td>82</td>
<td>75</td>
<td></td>
<td>19</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II II</td>
<td>110</td>
<td>75</td>
<td></td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Lesher</td>
<td>I</td>
<td>17</td>
<td>10</td>
<td>1</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>41</td>
<td>36 changed</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>41</td>
<td>36 changed</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blaschko</td>
<td>Early with symptoms</td>
<td>41</td>
<td>36 changed</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mueller</td>
<td>II relapsing</td>
<td>15</td>
<td>1</td>
<td>7</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Fischer</td>
<td>II relapsing</td>
<td>28</td>
<td>0</td>
<td>10</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Reinhart</td>
<td>II</td>
<td>Not stated</td>
<td>45%</td>
<td>38%</td>
<td>22%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>7</td>
<td>4</td>
<td>1</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>17</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blaschko</td>
<td>Late with symptoms</td>
<td>11</td>
<td>9 changed</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pinkhauer</td>
<td>III</td>
<td>18</td>
<td>2</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pinkhauer</td>
<td>III</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citron</td>
<td>Cerebral</td>
<td>33</td>
<td>15</td>
<td>12</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Blaschko</td>
<td>Late with symptoms</td>
<td>12</td>
<td>18 changed</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pinkhauer</td>
<td>Late with symptoms</td>
<td>12</td>
<td>18 changed</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blaschko</td>
<td>Latent late</td>
<td>15</td>
<td>13 changed</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pinkhauer</td>
<td>Latent late</td>
<td>15</td>
<td>4</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lesner</td>
<td>Latent</td>
<td>12</td>
<td>5</td>
<td>4</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Latent</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Citron</td>
<td>Latent</td>
<td>23</td>
<td>12</td>
<td>5</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Heller</td>
<td>Not stated</td>
<td>22</td>
<td>17</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hochne</td>
<td>Not stated</td>
<td>211</td>
<td>92</td>
<td>25</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td>Schönnefeld</td>
<td>Not stated</td>
<td>13</td>
<td>5</td>
<td>1</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Alt</td>
<td>General paresia</td>
<td>31</td>
<td>7</td>
<td>1</td>
<td>7</td>
<td>20</td>
</tr>
<tr>
<td>Alt</td>
<td>Epilepsy</td>
<td>6</td>
<td>4</td>
<td></td>
<td></td>
<td>2</td>
</tr>
</tbody>
</table>

The influence of the various forms of mercurial treatment is shown by the following table of Swift:
Effect of Mercurial Treatment on the Wassermann Test

<table>
<thead>
<tr>
<th>Number of cases</th>
<th>Bichlorid Injections</th>
<th>Biniiodid Injections</th>
<th>Salicylate Injections</th>
<th>Inunction</th>
<th>Bichlorid Pill</th>
<th>Mixed Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>3</td>
<td>7</td>
<td>14</td>
<td>17</td>
<td>10</td>
<td>28</td>
</tr>
<tr>
<td>Weak</td>
<td>66%</td>
<td>30%</td>
<td>21%</td>
<td>18%</td>
<td>20%</td>
<td>14%</td>
</tr>
<tr>
<td>Unaltered</td>
<td>70%</td>
<td>50%</td>
<td>24%</td>
<td>20%</td>
<td>50%</td>
<td>32%</td>
</tr>
<tr>
<td>Stronger</td>
<td>33%</td>
<td>8%</td>
<td>21%</td>
<td>58%</td>
<td>10%</td>
<td>14%</td>
</tr>
</tbody>
</table>

In this table, the biniiodid injections have the most beneficial result.

While it is clear that a persistent positive test indicates the necessity for further treatment, the finding of a negative reaction does not permit of a cessation of treatment indefinitely, as relapses have been reported after negative reactions have been brought about by treatment. Boas (9) has shown that such relapses are preceded by a return of the reaction.

**Treatment with Salvarsan.**—In a series of cases studied by Noguchi the time of the disappearance of the Wassermann reaction was as follows: As a rule, the reaction was much more slowly influenced than the symptoms of the disease, though clinical improvement was usually followed by a weakening or disappearance of the reaction. The earliest disappearance of the reaction in 34 cases was in two weeks, 1 case in the primary stage and 2 cases in the secondary stage. In the third week 10 cases became negative, 1 primary, 3 secondary, 3 tertiary, and 2 latent. In the fourth week 2 cases of primary syphilis, 5 secondary, and 4 tertiary cases became negative. One secondary case disappeared after 7 weeks. Craig (20) found that the percentage of disappearance of the reaction according to stages was as follows:

- **Primary syphilis** 80.6 per cent. became negative
- **Secondary syphilis** 74 “ “ “
- **Tertiary syphilis** 54.9 “ “ “
- **Latent syphilis** 72 “ “ “

The time at which the reaction disappeared, according to stages, was: tertiary mostly in 2 weeks; primary about half in 2 weeks; secondary from the second to the eighth week.

Craig also found that combined mercurial and salvarsan treatment gave a higher percentage of disappearance of the test than salvarsan and that fewer relapses occurred.

**Complement-Fixation in Gonococcal Infections**

Mueller and Oppenheim in 1906 (65) described a complement-fixation reaction obtained with the blood of a patient with gonorrheal arthritis. The control with a patient free from gonorrheal infection was negative. Since this work the method has been developed and come into wider and
wider use. According to Teague and Torrey (62), there are several different types of gonococci, immunization with one of which yields antibodies which fix complement best with their homologous antigen, that is, antigen from the same strain as was used in the production of antibody. The difficulty experienced by most observers has been that of obtaining an efficient antigen, as might be expected from the work of Teague and Torrey. The technique of the reaction which follows is that described by Schwartz and McNeil (76), which has been found valuable by a number of observers:

**Preparation of Antigen.**—The gonococci are grown on salt-free veal agar neutral in reaction to phenolphthalein. Twenty-four-hour cultures are washed off in distilled water and the resulting suspension heated for 2 hours in the water bath at 56° C. It is then centrifuged and passed through a Berkefeld filter. It is desirable to use a dozen or more strains in the preparation of antigen. The filtered fluid is then sealed in tubes and heated to 56° C. for 10 minutes on three successive days. As the antigen is needed, sufficient 9 per cent. salt solution is added to make the solution .9 per cent. salt solution strength. O'Neil finds that it is of advantage to allow the gonococcus suspension to autolize for several days in the ice chest before heating and filtering it. Recently Warden and Schmidt (85) have advocated an absolute alcohol extract of gonococci as an antigen giving a higher per cent. of positive tests than other antigens.

The employment of lipoid antigens in other diseases has met with the difficulty of positive reactions in syphilis (see page 141). Kohmer (52) has found the alcoholic extracts of little value, the best results being obtained with simple aqueous suspensions. Schwartz and McNeil have obtained good results with Parke, Davis & Co. gonococcus antigen.

**Technique.**—The technique of the test is analogous to that of the Wassermann reaction. The procedure is as follows:

**Titration of Amboceptor.**—A row of hemolysis tubes is arranged in a rack, and into each tube is placed 0.5 c. c. of 10 per cent. complement. Then into the different tubes are placed varying amounts of anti-sheep rabbit serum and the total quantity of each tube is made up to 1.5 c. c. To each tube 0.5 c. c. of 5 per cent. suspension of washed sheep erythrocytes is added, thus making the total volume 2 c. c. in each tube. The tubes are then incubated for an hour, and the lowest amount of amboceptor which causes complete hemolysis noted. This is called the unit of amboceptor, and twice this amount is used for the subsequent work.

**Titration of Antigen.**—A double row of tubes is arranged in a rack, and into each of the back row is placed 0.1 c. c. of serum from a normal individual free from gonococcus infection. Into each of the tubes of the front row is placed the same amount (0.1 c. c.) of serum from a known positive case of gonorrhea. In case a strongly positive serum is not obtainable an antigonococcic serum for therapeutic purposes may be substi-
tuted. Then into each consecutive pair of front and back tubes are placed increasing quantities of gonococcus antigen. The quantity of fluid in each tube is then made up to 1 c.c., and 0.5 c.c. of 10 per cent. guinea-pig serum is added to each tube. The set is then incubated for one hour, and then to each tube are added 0.5 c.c. of 5 per cent. suspension of sheep's erythrocytes and two units of amboceptor. At the end of an hour's incubation of the set of tubes, as now made up, the smallest amount of antigen is noted, which causes complete inhibition of hemolysis with gonorrheal serum, but which does not interfere with hemolysis when used with the normal serum. This is the unit of antigen. An efficient antigen should have considerable difference between the amount which will inhibit hemolysis with gonorrheal serum and that which will inhibit hemolysis alone or with normal serum. If many times the unit of antigen fails to inhibit with normal serum two units of antigen may be used for the test.

The Carrying Out of the Test.—After estimation of the antigen and amboceptor to be used, the test is carried out as follows:

<table>
<thead>
<tr>
<th>Tube Number</th>
<th>Patient's Serum c.c.</th>
<th>Normal Serum c.c.</th>
<th>Positive Serum c.c.</th>
<th>Antigen Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.05</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>0.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0.05</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>0.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0.05</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>0.1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>0.05</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>0.1</td>
<td>0.05</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0.05</td>
<td>2</td>
</tr>
<tr>
<td>11</td>
<td>0</td>
<td>0</td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>0</td>
<td>0</td>
<td>0.1</td>
<td>0</td>
</tr>
</tbody>
</table>

The tubes are then made up to 1.5 c.c. and incubated for one hour. At the end of this time 2 units of amboceptor and 0.5 c.c. of 5 per cent. suspension of sheep's cells are added and the test incubated for a second hour. At the end of this time, in a positive reaction, tubes 1 and 2 and tubes 9 and 10 should contain no hemolysis, while the others should be completely hemolyzed. Instead of the sheep system, the Noguchi human system, as described in connection with the Wassermann test, may be used.

Significance of the Test.—In contrast to the Wassermann test the complement-fixation reaction in gonorrhea is biologically specific, and depends upon so-called specific, antigen antibody interaction.

Clinical Value of the Tests.—The reaction is not to be expected earlier than the fourth week of the disease, and only in acute cases with complication as acute prostatitis or arthritis. The reaction persists usually for seven or eight weeks, and a persistence of the reaction longer than this may indicate that the patient still harbors gonococci. The value of the test in the various forms of gonococcal infections is as follows:
Acute Anterior Urethritis.—Usually a negative reaction is obtained according to Schwartz and McNeil. It is in these cases that bacteriological diagnosis is least difficult.

Chronic Posterior Urethritis, Prostatitis, Vesiculitis, and Stricture.—O'Neil found 50 of 60 cases with a positive reaction.

Gynecological Cases.—Infections of the Lower Genital Tract.—O'Neil's cases showed 21 positive reactions in 24 cases.

Pelvic Infections.—Twelve cases known to be gonorrheal in origin gave positive tests. According to Schwartz and McNeil, a positive reaction may not be expected without involvement of the cervix uteri.

Arthritis.—O'Neil found in 14 cases of arthritis with gonorrhea or gonorrheal history 11 positive reactions. In 6 cases with doubtful history 3 were positive and 3 negative. In 5 cases with a negative history 1 case was positive and 4 negative.

Vulvovaginitis in Children.—McNeil (63) found a positive reaction in 8 cases, some of which had no evident discharge. Kolmer (52) states that 60 per cent. of all gonorrheal cases give positive tests. The highest per cent. (83 per cent.) of positive tests is obtained in gonorrheal arthritis.

According to all observers, control cases in which gonorrheal infection can be excluded give a negative reaction, so that the test is strictly specific for gonococcus infections. Schwartz and McNeil were able to obtain a positive test with antimumingococcic serum, but not with the serum of patients with epidemic cerebrospinal meningitis.

Complement-Fixation in Echinococcus Disease and Other Tenias

The complement-fixation test has been a valuable aid in diagnosis in the hands of a large number of observers. The technique of the reaction is so similar to the complement-fixation tests in syphilis and gonorrhea that only the points of difference will be entered into in detail.

Preparation of Antigen.—There is considerable difference of opinion as to the best method of preparing antigen for the test. Antigens have been made of alcoholic and aqueous extracts of the wall of echinococcus cysts and from the cyst fluid. According to Hahn (35), watery extracts of the cyst wall yield the most efficient antigen. Hahn finds that lipoid extracts serve as antigens, but that with such antigens a positive reaction is obtained with the serum of syphilities. Either the cysts from human beings or from swine may be used. Thomsen and Magnussen (79) obtain the best results with the unaltered fluid from the cysts, and find that it keeps well for at least six months.

Technique of the Test.—The technique of the test consists of the titration of the indicator system (complement amboceptor and sheep cells), as described for the Wassermann test. The titration of the antigen and
the test proper are performed by a technique similar to that used in the Wassermann test for syphilis.

Clinical Value of the Test.—All observers are agreed that the test is of great value in diagnosis. The percentages obtained by most observers agree with the figures of Thomsen and Magnussen, who found 10 out of 12 cases positive. The reaction with watery extracts or cyst fluid is specific for echinococcus, according to Thomsen and Magnussen. Meyer (64) and Hahn, on the other hand, regard the reaction as a so-called group reaction and obtain positive tests with the serum of patients with tapeworm. In the same way antigens of tenia give a positive test with the serum of both echinococcus and other tenias.

Complement-Fixation in Tuberculosis and Other Diseases

The presence of complement-binding antibodies in the blood of tuberculous patients has been observed by many writers. Some of these have advocated the reaction as a valuable means of diagnosis. There have been many different methods of antigen preparation and there seems little doubt that specific reactions are obtainable by some of these methods. [See résumé by Korper (53).] One difficulty with this and other immune reactions in tuberculosis is well stated by Hamman (37), who reminds us that the prevalence of tuberculous infection makes it necessary for the clinician to distinguish between the presence of tuberculous infection and tuberculous disease, a distinction which in other diseases, as syphilis, is not necessary. According to Ludke and also Loewenstein (56), the reaction is found more frequently in those patients who have been treated with tuberculin.

The complement-fixation phenomenon has been observed in a great number of diseases, but has not been of any great diagnostic value in most of them. In typhoid fever, for instance, the complement-fixation test may be obtained, but appears to offer no advantage over the much simpler Widal test. The reaction is also used in order to ascertain the presence of antigens in the body of the patient, as, for instance, the products of tubercle bacilli in the blood of patients with miliary tuberculosis. The procedure has not, however, been of practical value.

The Immune-ferment Reactions (Abderhalden)

Following the observation of the phenomenon now known as anaphylactic shock, extensive investigations were made in order to find the nature of the change in an animal by which it was rendered hypersusceptible to protein materials. In 1906 Vaughan (82) advanced the theory that anaphylactic shock is due to an intoxication with protein-split products and that the condition of hypersusceptibility is due to an increased protein-splitting power of the blood. This view of Vaughan has been supported by an enormous mass of evidence. It remained for Abderhalden and his
collaborators to make an accurate study of these protein-splitting ferments of the blood and their relation to immunity and disease. In order to study the specificity of these ferments, Abderhalden and Pincussohn (2) injected gelatin into dogs and found that the ferments which subsequently developed split not only gelatin but peptones from silk and other sources. With others Abderhalden (1) showed that injection of peptones from various sources gave rise to ferments which were capable of splitting not only the peptones which brought about their formation, but also peptones from other sources. An exception to this rule was found in peptone from placenta, which gave rise to specific ferments which acted only upon peptone from placenta. Using unsplit proteins as antigen, Dick (25) was able to demonstrate the production of ferments which were specific for the antigens employed and which were very similar to the other immune antibodies such as lysins. It would be expected that such immune ferments would develop in the blood of such animals as received through infection or other conditions a parenteral introduction of protein substances. Schmorl (75) and others showed that chorionic epithelium enters the maternal circulation during pregnancy. Abderhalden was able to demonstrate that ferments having the power to split protein from chorionic epithelium (placenta) were present in the maternal blood during pregnancy.

Dick (26) demonstrated the presence of proteolytic ferments in the blood of pneumonics. The ferment action was first demonstrated by Abderhalden by means of the so-called optical method. This method depends upon the fact that solutions of peptones have a capacity for rotating a beam of polarized light which varies from that of their split products. Thus, if a serum containing ferments is mixed with a solution of peptone and the degree of optical rotation estimated before and after the proteolysis has occurred, a difference is found in the degree of optical rotation.

On account of the difficulty of carrying out the technique of the optical method the dialyzation method was afterward used. This method of demonstrating that protein-splitting has taken place depends upon the fact that unsplit protein material does not pass through certain membranes, while the proteolytic products of these proteins, such as the amino acids, which are crystalloids, may pass through the same membranes. The presence of peptone was first tested for by means of the well-known biuret reaction. Afterward the substance ninhydrin (triketohydrindenhydrate) was used as a reagent to demonstrate the presence of either peptone or amino acids. The most important application of Abderhalden’s work to diagnosis has been his test for pregnancy.

**Serodiagnosis of Pregnancy**

Owing to the difficult technique, the optical method with the placental peptone as antigen has been but little used.
Dialyzation Method.—Preparation of Placental Albumin.—A normal fresh placenta is freed from membranes and umbilical cord. It is then cut into pieces about the size of a twenty-five-cent piece and these pieces washed in running water until they are free from blood. The tissue is then bailed in two liters of water containing two drops of glacial acetic acid. This is best done in a large porcelain basin. When the tissue has boiled for 5 minutes the water is changed and boiling continued for 5 minutes more. After the second boiling the water is tested for protein-split products (peptone and amino-acids) by the ninhydrin test as follows: 10 c. c. of the water is filtered into a large test tube and 1 c. c. of a 1 per cent. solution of ninhydrin added. The mixture is boiled one minute from the time at which bubbles begin to appear in it. The tube is then allowed to cool. If at the end of one-half hour the mixture is colorless, the test is negative. The presence of peptone or amino-acids will be shown by a coloring of the solution from a slight lilac tinge to a violet, depending on the intensity of the reaction. If the test is positive the water is changed again and the placenta boiled as before. This process is repeated as long as a positive ninhydrin test is obtained. Thorough washing in running water before boiling facilitates the removal of protein-split products. A stock of peptone-free and amino-acid-free placenta prepared in this way may be kept in chloroform water under toluol. Jellinghaus and Losse (45) find the following measures of value in obtaining an efficient antigen: (1) The placenta is cut up and washed in salt solution immediately upon expulsion, as the minimum amount of clotting has taken place at this time. (2) The boiled tissue should be teased in uniformly small threads. (3) Aseptic technique is carried out as far as possible.

Obtaining the Serum.—The serum is obtained as described for immune reactions in general. It is especially desirable to obtain the blood from the patient before meals, as after ingestion of food amino-acids may be present in the blood.

Partial taking of the blood should be avoided. From 12 to 15 c. c. of blood is sufficient for the test and controls.

Testing the Dialyzers.—Dialyzer No. 579 A, made by Schleicher and Schuell, should be used. These dialyzers should be tested before use as follows: (1) They should permit the passage of peptone. In a number of dialyzers to be tested are placed 5 c. c. of a 1 per cent. solution of “seiden peptone Farbewerke Hoechst.” The dialyzers are then placed in test tubes of such a size that about 0.25 cm. space exists between the inner wall of the test tube and the dialyzer. The test tubes contain 20 c. c. of distilled sterile water. The container and dialyzer are then incubated for 18 to 24 hours, and after removal of the dialyzer the dialysate in the test tube is tested with 0.2 c. c. of 1 per cent. ninhydrin for the presence of peptone. Only those dialyzers are selected which give a moderate shade of color with the test, and those which are faintly tinged or give a deep color are discarded. These
dialyzers are then washed, boiled, and tested for permeability to albumin in a manner similar to that of the test for permeability to peptone, except that 5 c. c. of serum or egg-albumen replaces the peptone solution, and the dialyze is tested after 24 hours for albumin. Those filters which are found permeable to peptone and impermeable to albumin are washed, boiled, and kept in chloroform water covered with a layer of toluol. The dialyzers may be used repeatedly if properly tested.

Technique of the Test.—A quantity of stock placental tissue prepared as described is again tested for protein-split products as described by means of ninhydrin. A tested dialyzer is washed and boiled, and one gram of placenta free from dialyzable substances is placed in the bottom of the dialyzer, and 1.5 c. c. of the test serum and 2 drops of toluol added. The top of the dialyzer is then held together and the outside washed in running tap water. The dialyzer is then placed in a test tube such as was used in testing the dialyzer and containing 20 c. c. of distilled water covered with a layer of toluol. A cotton plug is inserted into the mouth of the dialyzer, and care is taken that the water in the tube extends slightly above the level of the serum within the dialyzer. The dialyzer is incubated at 37° C. for 16 hours. At the end of this time the dialyzer is removed from the dialysate and 10 c. c. of the dialysate taken from beneath the toluol with a pipette is placed in a small breaker or another test tube and 0.2 c. c. of 1 per cent. ninhydrin added. As soon as the mixture bubbles, it is boiled for one minute and the color is noted after it has cooled for one-half hour. A positive reaction results in a color from a light lavender tinge to a bluish purple. A negative reaction is indicated by a colorless or more commonly a slight yellow tinge. Controls should be made by carrying out exactly the same procedure with the following exceptions. In control 1 test serum heated to 60° C. for 10 minutes is substituted for active serum in order to make sure the dialyzable substances do not exist performed in the serum. In control 2 distilled water is substituted for serum in order to make sure that the placental tissue is free from dialyzable substances. A third control using a known positive serum may also be made to be sure that all conditions are favorable for the test. To guard against error two test mixtures may be made instead of one. If a slight reaction in the negative controls is accompanied by a more marked reaction in the test mixture, a positive diagnosis is permissible.

Value of the Reaction.—Abderhalden obtained a positive reaction as early as six weeks from the first day of the last menstruation and as late as fifteen days after the termination of pregnancy. Abderhalden failed to find the reaction in non-pregnant individuals. The results of the test in the hands of other observers have varied widely. The thorough quantitative work of Van Slyke, Vinograd, Wilchur and Losee (81) has shown that although the serum of pregnant individuals averages higher in proteolytic power than that of nonpregnant individuals, the range of proteolytic power
of pregnant and nonpregnant sera is so nearly alike that the test is of little practical value.

**Abderhalden Test for Carcinoma**

Following the introduction of the test for pregnancy by means of the dialysis method, Abderhalden, by the substitution of carcinomatous tissue instead of placenta, originated a test for carcinoma which promises to be of value. The technique is the same as that for pregnancy, with the exception that carcinomatous tissue is used for antigen and is prepared in the same way as the placental tissue. Using this technique, Frank and Heiman (31) obtained positive reactions in 53 of 54 cases of carcinoma. Gambaroff (32) obtained a positive test in 49 of 50 cases. He was able also to differentiate carcinoma and sarcoma by means of the method. Brockman (11) also confirms the results of others, and found that carcinoma from any source might serve as antigen for testing for carcinoma of other organs. Brockman obtained positive reactions in 100 per cent. of his 25 cases and negative in all of the controls. The results of Dick (27), Van Slyke (86), and others indicate that the test is of little value.

**The Ferment Reaction in Other Diseases**

The ferment reaction has been used by Abderhalden and others to ascertain the presence of a variety of intoxications, including infections, thyroid disease, and insanity. In order to ascertain the presence of intoxication by the absorption of thyroid proteins, for instance, the blood is tested for the presence of ferments having the power of splitting thyroid protein. It is not likely that these reactions will be of clinical value.

**The Miostagmin Reaction**

The miostagmin reaction depends upon the principle that, through antigen and antibody interaction, changes occur in fluids containing them, which are accompanied by a lowering of the surface tension. This change in surface tension is determined by counting the number of drops which a certain volume of fluid will yield as dropped from an instrument known as a stalgmometer. The lower the surface tension becomes through the interaction of antigen and antibody, the greater the number of drops yielded by a given volume. When, therefore, antigen and antibody, which are biologically specific, are mixed together a given volume will yield a larger number of drops after the interaction has been completed than before. When the antigen and antibody are not biologically specific no change of surface tension occurs, and the number of drops remains the same after interaction has taken place as before it has begun.
The reaction has through the work of Ascoli and Izar and others been used in the diagnosis of a great number of diseases. Only those in which the procedure has been found most valuable will be mentioned.

**The Miostagmin Reaction in Carcinoma**

**Preparation of Antigen.**—The difficulty or preparation of an efficient antigen is a point upon which all observers concur. Burmeister (14) used with satisfactory results the technique of Ascoli and Izar (4), substituting pancreas for tumor tissue, as it was found that neither carcinoma nor other tissues yielded as good results as pancreas. The antigen was then prepared by drying the tissue, ground with sand, in a current of warm air. The powdered desiccate was treated with methyl alcohol in the proportion of 1:4 at 50° C. for 24 hours, with frequent shakings. This was then filtered while hot through a Schleicher and Schuell filter No. 590. After cooling the filtrate was again filtered as before. The extract was then titrated in watery emulsions with normal serums until a dilution of antigen was obtained that would not cause an increase of more than one drop with Traube's stalagmometer.

**Technique of the Test.**—Mixture 1, 9 c. c. of test serum diluted 1-20 with normal salt and 1 c. c. of distilled water. Mixture 2, 9 c. c. of diluted test serum, 1 c. c. of antigen emulsion. Mixtures 3 and 4 may be made as additional controls, using known positive and known negative control serums instead of the test serum. The number of drops yielded by a given volume is estimated in each mixture at once and after incubating for one hour in a water bath at 50° C. If in the test mixture the number of drops after incubation exceeds that obtained before incubation by two or more, the reaction is considered positive. The number of drops increase to be considered positive has been varied by different experimenters.

**Value of the Test.**—The estimation of the value of the test varies most widely with different experimenters. Most observers find practically all negative cases give negative reactions. Others, however, find as high as 20 per cent. of non-carcinomatous individuals who give a positive test. In known carcinoma cases positive reactions are reported by different observers as from 47 to 96 per cent. positive, a wide variation in results. Burmeister concludes that a negative reaction is of more value than a positive one and that strongly positive reactions are not necessarily indicative of carcinoma. Kelling (50) and Burmeister warn against leaving any soap on the glassware.

**The Miostagmin Reaction in Tuberculosis**

A number of observers have used the miostagmin reaction in the diagnosis of tuberculosis. Izar (44) prepared antigen for the test as follows:
Tubercle bacilli are ground in a mortar with 96 per cent. alcohol and the mixture incubated several days at 37° C. with frequent renewal of the alcohol until the supernatant alcohol remains clear. The bacillary residue is then dried at 47° C. and extracted with alcohol and then with ether. The collected alcoholic extract is dried at 47° C., the ether extract is added to the residue, and the whole evaporated at 30° C. The residue dissolved in absolute alcohol is filtered and evaporated until precipitation begins to show. The solution is then filtered and ether added drop by drop until a precipitate begins to form. This is then removed by filtering through a No. 417 Dreverhoff filter and the ether evaporated. More ether is then added until a precipitate again forms and the precipitate is again removed as before. The filtrate is then evaporated to dryness and dissolved in absolute alcohol. This filtrate evaporated to saturation constitutes the antigen. Using a technique similar to that described for carcinoma, Izar obtained a positive reaction in 34 of 35 cases of known tuberculosis in which no tubercle bacilli were found in the sputum. The reaction is so specific, according to Gasbarrini (33), that bovine tuberculosis may be distinguished from the human type.

The Miestagmin Reaction in Other Diseases

In echinococcosis disease Izar (43) obtained good diagnostic reactions, using directly the cyst fluid or alcoholic extracts of it for antigen. Inasmuch as we have in the other diseases, which have been found to give diagnostic miestagmin reactions, other simpler means of diagnosis, it may simply be mentioned that the reaction has been used in syphilis, typhoid fever, etc.

The Epiphnin Reaction

The epiphnin reaction makes use of the principle that the absorptive power of the surface of fine particles in suspension is changed in the presence of a specific antigen-antibody reaction. For demonstrating this change a barium hydrate, sulphuric acid system is employed. In this system the sulphuric acid is of such concentration as to exactly neutralize an equal volume of saturated barium hydrate solution. A definite quantity of barium hydrate and sulphuric acid is added to definite amounts of antigen antibody mixture. Equal quantities of barium hydrate and sulphuric acid are then added to antigen antibody mixture which has been incubated, and the degree to which the point of neutralization has shifted is found by titration with n/1000 H₂SO₄. For the purpose of an indicator a 1 per cent. solution of phenolphthalein in alcohol is used. As a catalyst a 10 per cent. solution of strontium chlorid is added.

The epiphnin reaction has been most used in carcinoma, and the technique of the test as applied to carcinoma will be given.
Preparation of the Antigen.—According to Rosenthal (73), tumor material is ground in a mortar with quartz in 20 per cent. glycerin water, 1 part of tumor tissue being used to 10 parts of glycerin water. The mixture is extracted from 3 to 24 hours and then centrifuged. The supernatant fluid is used as antigen.

Technique of the Test.—Burmeister (15) describes the technique of the test as follows: The titrations are carried out in a series of four beakers of equal dimensions. In beaker 1 are placed 0.1 c. c. of the serum to be tested and 1 c. c. of the antigen dilution. In beaker 2 are placed 1 c. c. of the antigen and 0.1 c. c. of distilled water. In beaker 3 are placed 1 c. c. of water and 0.1 c. c. of the serum to be tested. In beaker 4 are placed 1.1 c. c. of distilled water. The contents of the beakers are then mixed by gently shaking and then allowed to stand 10 or 15 minutes. Following this, 3 c. c. of saturated barium hydrate and 3 c. c. of sulphuric acid are added and 0.1 c. c. of a mixture of equal parts of 1 per cent. phenolphthalein in alcohol and 10 per cent. solution of strontium chlorid in water is added to each beaker. The contents of beaker 4 are then added to beaker 1 and those of beaker 3 to beaker 2. Beaker 1 and beaker 2 then contain equal quantities of reagents, indicator, water, antigen, and antiserum, the difference being that the antigen and antiserum of beaker 1 have had time to interact before the addition of the indicator system. If in beaker 1 more acid has been absorbed than in beaker 2, the extent of the difference is estimated by adding n/1000 H₂SO₄ until the color is the same as in beaker 2, which should be of a slight pinkish tinge. In the same way normal and test serums may be compared in various dilutions of 1-10, 1-100, and 1-1000, curves being made to illustrate the variations. According to Angerer and Stoelter (50), greater accuracy may be obtained by the use of only one beaker and one set of reagents and increasing the quantity of antigen and antiserum. Rosenthal finds the method a satisfactory one for the differentiation of carcinoma protein. Burmeister has found the test valueless as a means of diagnosis of carcinoma.

The Opsonins in Diagnosis

But little of the diagnostic value which was expected of the opsonic reaction has been realized. The reaction is, however, occasionally of some use, and the technique and significance of the reaction will therefore be briefly entered into. Opsonins are substances existing in the body fluids which are capable of rendering bacteria more susceptible to phagocytosis. When in an animal the body fluids have for any bacteria a high opsonic content, the resistance of the animal to infection with those bacteria is high. As a rule, a low opsonic content denotes a lowered resistance. The infection of an animal with any given bacterium will result in variation from the normal in the opsonic content either in the direction of an increase or
decrease, depending upon the success or failure of the immune processes in combating the infection. On this account a variation from the normal in opsonic content of a serum indicates the presence of an infectious process. If, for example, the serum of a patient is found to be high in opsonins against typhoid bacilli, it is likely that the patient has been or is the subject of infection with typhoid bacilli; if the serum of the patient is abnormally low in opsonins against typhoid bacilli, it is probable that he is unsuccessfully resisting a typhoid infection.

The opsonic content of a given serum is estimated by comparison with normal serum in either of two ways. (1) The opsonic index. By the opsonic index is meant the ratio of the average number of bacteria per leukocyte taken up in the presence of the given serum to the average number taken up in the presence of a normal serum. (2) The point of opsonic extinction. The point of opsonic extinction is the point of dilution of a serum at which it ceases to have an opsonic action.

**Technique of the Opsonic Index Estimation.**—The factors taking part in the opsonic reaction are: the serum to be tested, the normal serum with which it is compared, the leukocytic suspension, and the bacterial suspension.

The serum to be tested is obtained as described on page 117. A few drops are sufficient. Inasmuch as immune opsonins are resistant to a temperature of 56° C. for one-half hour, it is advisable to heat the serum in this way so as to exclude the action of normal opsonins which are thermolabile. The serum used as a standard is obtained from an individual in whom previous or present infection with the organism in question can be excluded. It is treated in exactly the same way as the serum in which the opsonic content is to be tested.

**The Leukocytic Suspension.**—The leukocytes are obtained by puncturing the lobe of the ear or the tip of the finger with a lancet and allowing 10 to 20 drops of blood to flow into a solution of 1 per cent. sodium citrate in 0.85 per cent. sodium chloride solution. The citrate solution keeps the blood from clotting until the corpuscles are separated from the citrate solution by centrifugation. The supernatant fluid, after the corpuscles have been sedimented by centrifugation, is removed by means of a pipette and the corpuscles washed by replacing the citrate solution with 0.85 per cent. sodium chloride solution and again centrifuging. After two such washings the pearly white layer of leukocytes which covers the erythrocytes is removed with a capillary pipette and used as the leukocytic suspension.

**Bacterial Suspension.**—The bacteria are grown on a suitable solid medium, and at the height of their growth are washed off the media by adding 0.85 per cent. sodium chloride solution and gently agitating the fluid or actually scraping off the growth with a platinum loop. The water of condensation should be previously removed. The suspension should be shaken sufficiently to insure breaking up clumps of organisms. The sus-
pension should be of such a concentration that in a smear on a slide stained by the ordinary methods bacteria are plentiful in each field, but the individual organisms remain well separated from one another.

The estimation is then carried out as follows: A number of pipettes are made by drawing out into capillaries small pieces of glass tubing with a lumen about 4 mm. in diameter and about 15 cm. long. The capillary should be about 20 cm. in length and of uniform diameter. The end of the capillary is allowed to touch the bacterial suspension, when the suspension will enter the tube by capillarity. When a column of an inch or so in length has entered the tube it is withdrawn from contact with the suspension and the length of this column accurately marked on the outside of the capillary with a paraffin pencil. A small air bubble is then drawn into the pipette and following this a volume of the serum to be tested, which equals the volume of bacterial suspension used. Another air bubble is drawn into the tube and then a volume of leukocytic suspension the same as that of bacteria and of serum. The three constituents are then mixed together on a clean slide, drawn back into the pipette, and incubated for 15 minutes. (The time of incubation should be such that the optimum phagocytosis is obtained with the minimum of digestion of the bacteria.) The mixture is then spread on a glass slide in the same way as a smear for differential blood counting is made and stained with a suitable stain. In most cases one of the eosinates of methylene blue gives good results. In the case of tubercle bacilli, for example, special stains must be used. A second preparation is made in exactly the same way as the one described, excepting that normal serum is used instead of the serum to be tested. The number of bacteria taken up by each leukocyte is then observed by means of the oil immersion objective and an average for 200 leukocytes or more computed. Then if, for instance, twice as many bacteria are taken up by the leukocytes in the test serum mixture as in the normal serum mixture, the opsonic index is 2. Care must be taken to use corresponding parts of the slides, as regards edges and center, in counting the test and normal slides.

The technique of estimation of the point of opsonic extinction is the same as for that of the opsonic index, except that instead of undiluted serum various degrees of dilution are used and the point found at which the serum does not influence phagocytability more than a control of salt solution. Combinations of the two methods may also be used. That is, the opsonic index at various dilutions may be determined. The comparative number of leukocytes taking part in phagocytosis is also considered of value, and has been called the phagocytic index.

Instead of the opsonic power of the blood, the phagocytic power of the patient's leukocytes with the patient's serum may be compared with the phagocytic power of normal leukocytes with normal serum.

**Clinical Value of the Opsonic Reaction.**—According to Wright and Bullock (91), the opsonic index in normal individuals varies between 0.8
and 1.2. The deviation of the index beyond these limits would indicate that the patient was or had been infected with the organisms to which the variation in the index existed. These variations in the opsonic index have not been found of much practical value.

**DIAGNOSTIC REACTIONS OCCURRING IN THE PATIENT**

The diagnostic immune reactions which occur in the body of the patient belong to that class of phenomena which has been termed by von Pirquet allergy. By allergy is meant an altered power of reaction to foreign proteins. When a person has been subjected to the influence of agents such as pathogenic micro-organisms and their products his power of reacting to those organisms or their products becomes changed from the normal, and this change has been utilized in diagnosis. Accordingly as the patient is immunized against a preformed toxin or merely sensitized to a substance not in itself a toxin, there are two types of allergic reactions occurring in the body of the patient:

1. In which preformed toxin is used as antigen,—a positive reaction indicates a lack of immune substances. The Schick reaction is the only test of this type in use and as it is not strictly a diagnostic test it is not discussed here.

2. Reactions in which the nontoxic antigen must be converted into toxin by anaphylactic antibodies. It is this type of reaction which has been of most value in diagnosis.

Inasmuch as it is of more interest to consider the relation of one kind of reaction in a particular disease to another variety of reaction in the same disease, the various reactions in different diseases will be considered together.

**Tuberculosis**

*The Subcutaneous Tuberculin Test*

Of the allergic reactions the first to be used to any great extent was that of the subcutaneous tuberculin test of Koch. Koch observed that, when healthy guinea-pigs were injected subcutaneously with suspensions of tubercle bacilli, the injection was followed by only a slight local reaction. The subcutaneous injection of even smaller amounts of suspensions of tubercle bacilli in guinea-pigs, which were the subject of experimental tuberculosis, was followed, on the other hand, by severe local disturbance and subsequent death of the animal in from 6 to 48 hours. In cases where non-lethal doses of bacilli were used severe necrosis of the skin occurred at the site of injection, an occurrence now known as “Arthus’ phenomenon.” The observation that tuberculous patients were much more susceptible to tu-
berculin than normal individuals was soon applied to the diagnosis of tuberculosis.

**Nature of Tuberculin.**—The readiness with which Koch's old tuberculin is obtainable makes a detailed description of the preparation unnecessary. The product consists of sterilized filtered growths of tubercle bacilli in glycerin broth. This filtrate, containing the products of the growth of the tubercle bacilli, is standardized by finding the amount necessary to kill guinea-pigs which have been infected with tuberculosis. By concentration or dilution the preparation is in this way made of the same strength as a standard preparation.

**Effect of Tuberculin on Healthy Individuals.**—The effect of lethal amounts of tuberculin on healthy persons is described by Koch (51), who infected himself with 250 mg. In a few hours he began to have difficulty in breathing, a tendency to cough, marked malaise, and in 5 hours a chill occurred, which lasted about an hour. At the same time there was vomiting, and the temperature rose to 100°. The symptoms lasted about 12 hours and then began to subside. By the next day only a condition of fatigue remained. Except in such an enormous dosage, tuberculin is without effect on non-tuberculous individuals. The usual limit of tolerance of non-tuberculous individuals is about 10 mg. Loewenstein obtained in 8 out of 10 normal persons a slight febrile reaction with 10 mg. of tuberculin.

**Technique of the Test.**—**Preparation of the Patient.**—Before making a tuberculin test the patient should be carefully observed for two days to one week. Observation should include a record of the temperature taken every two hours, careful examination and record of the condition of any suspected lesions, including such items as the amount of sputum and its character, the physical signs, etc. The general condition of the patient should be inquired into in order to exclude severe cardiac renal or other disease.

**Injection of the Tuberculin.**—Tuberculin is prepared so that 1 c. c. of the liquid contains approximately 1 gm. of tuberculin, and it is important to obtain accurate sterile dilutions of this fluid. The diluting fluid consists of 0.5 per cent. carabolic acid and in an 85 per cent. sodium chloride solution. The solution may be made up in a flask, and is best sterilized in an autoclave, but may be sterilized by boiling for one-half hour. If boiling is the method selected about 100 to 200 c. c. of water should be added to a liter to allow for evaporation. One-ounce glass-stoppered bottles serve as containers for the dilutions. The bottles may be sterilized in the autoclave or by heating to 200° C. for 10 minutes. With a sterile 1 c. c. pipette marked with 0.01 c. c. graduations 0.1 c. c. of tuberculin is measured into one of the bottles and 9.9 c. c. of the diluting fluid added. In this way a dilution of 1-100 is obtained. This may be labeled dilution 3. (Dilution 2 or 1-10 is too strong for diagnostic purposes and need not be made.) Dilution 3 then will contain 10 mg. to 1 c. c. To 1 c. c. of dilu-
tion 3 in another bottle are added 9 c. c. of diluting fluid. In this way dilution 4 of 1-1000 is obtained in which 1 c. c. equals 1.0 mg. These dilutions may also be made by adding to 1 drop of tuberculin 10 drops of diluting fluid, etc., but the method is neither as accurate nor as rapid as the metric measurements. The injection is made with a sterile syringe of the Luer or similar type, which will measure drops of 0.1 c. c. The injection is made under the skin of the back below the angle of the scapula or in the arm in the region of the insertion of the deltoid muscle. The skin may be sterilized by washing with 50 per cent. alcohol.

The Dosage of Tuberculin.—The dosage of tuberculin to be used in diagnosis has been the subject of much discussion. Koch (51) recommended an initial injection of from 0.1 to 10 mg., depending upon the condition of the patient. In weak individuals 0.1 mg. was used, while in comparatively healthy strong persons 1 mg. was injected. If there was no reaction in 48 hours, double this dose was used. If there was still no reaction within 48 hours following this, a third injection of 5 mg. was given. If, however, a rise of temperature of a degree or less occurred, the third injection was made the same as the second. Koch regarded 10 mg. as the limit at which a reaction was to be taken as specific. Others, however, have recommended as high as 50 mg. before concluding that the reaction is negative. Loewenstein and Rappaport (57) recommended an initial dose of 1/5 mg. repeated every third day until a reaction is obtained or until four doses have been given. It is claimed for this reaction that the small initial dose gives a more specific test and that with the four repeated injections no cases of tuberculosis will fail to react. Hamman and Wolman (31), however, find that a number of cases in which a positive diagnosis is certain fail to react with this method and recommend increasing doses beginning with 1/5 mg. first, 1 mg. second, ending with 5 mg. as a third dose. Baudelier and Roepke (6) recommend four doses of 1/5 mg., 1 mg., 5 mg., and 10 mg. The method of Hamman may be carried out by injecting as an initial dose 0.2 c. c. (3 drops) of dilution No. 4, as described above, and using as a second dose 1 c. c. (15 drops), and ending with 1/2 c. c. (7 drops) of No. 3 dilution.

The positive tuberculin reaction is followed by changes at the site of injection (local reaction), changes at the foci of tuberculous processes (focal reaction), and constitutional disturbances.

Local Reaction.—In a few hours after the injection swelling occurs at the site of injection, which is accompanied by pain and reddening of the skin. In 8 to 12 hours a swelling is felt which may reach a size of several centimeters in diameter. After persisting for a period of from two days to a week the local reaction subsides. Usually it has disappeared by the end of the week.

The Focal Reaction.—The focal reaction is best observed in the cases in which the tuberculous lesion is externally located. The lesions of
lupus, for example, become swollen and a surrounding area of hyper-emia develops. Tuberculous glands become swollen, red, and painful. In pulmonary lesions the change may be noted by an increase in the number of râles heard over the area, an increase in the quantity of sputum may occur, and, according to some observers (Loewenstein, 56), tubercle bacilli may appear in a sputum in which they were formerly absent. According to Brown (12), this is a rare occurrence.

The General Reaction.—The constitutional symptoms occurring in a positive tuberculin test begin in from 8 to 16 hours after injection, as a usual thing, depending somewhat on the dose employed. Delayed reactions sometimes occur as late as 48 hours after injection. The subjective symptoms consist of malaise, headache, and tendency to cough; nausea and vomiting may occur and chills of varying severity are common. Accompanying these symptoms there is a rise of temperature which is most important from the diagnostic standpoint. This rise in temperature may be of insignificant grade or may reach 104° F. There has been considerable discussion as to what shall be considered sufficient evidence upon which to base a positive diagnosis. Any definite designation must, of course, be more or less arbitrary, but a rise of 1° F. or more above the maximum temperature before the test is regarded by Hamman and Wolman (36) and others as positive. This selection is based upon confirmatory evidence of the presence of a tuberculous process.

The Diagnostic Value of the Reaction.—There has been the widest variation in the estimates of the value of the subcutaneous tuberculin reaction. In considering the worth of the test it should be remembered that the tuberculin reaction is evidence of a sensitization with products of tubercle bacilli, and that such sensitization does not vary directly as the activity of the process, but that old healed tuberculous foci may result in marked hypersensitiveness to tuberculin. The well-known figures of Naegeli (66), who found anatomical evidence of tuberculous processes in 97 per cent. of autopsies, will emphasize the point that a high percentage of healthy individuals may be expected to be hypersensitive to tuberculin. As a matter of fact, Hamman and Wolman estimate from their own experience and that of others that from 40 to 60 per cent. of persons with no active tuberculous lesions react to tuberculin. It will be seen then that, as an indication of the presence of an active tuberculous lesion, the tuberculin test is to be considered only as confirmatory or additional evidence. The reaction is specific, however, and a negative reaction is of great value in excluding tuberculosis.

Contraindications and Dangers.—The most important contraindication is the presence of a temperature of any considerable extent. Where the control temperature runs above 99.6° F., and especially where the temperature is irregular, the rise after a tuberculin injection cannot be definitely connected with the tuberculin. Again the intoxication present in
such cases may result in a condition of anti-anaphylaxis, so that the patient, even though tuberculous, may fail to react. There is a widespread hesitancy about the use of tuberculin on account of the focal reaction which has been described. This reaction is feared as a cause of the dissemination of the tuberculous process. The opinion of those who have had the largest experience with tuberculin injections, however, is almost universally reassuring, and there seems reason to believe that some of the extensions of tuberculous processes which have been reported have been coincidences. Among other contraindications are mentioned organic heart disease, pregnancy, tuberculosis of the larynx, diabetes, and nephritis.

The Cutaneous Tuberculin Test (von Pirquet)

The difference between the reaction of vaccinated individuals and unvaccinated to vaccination was observed by Jenner. No use, however, was made of this principle until v. Pirquet made a study of the same phenomena and later applied the idea to a cutaneous tuberculin test. He found that infection with tuberculosis resulted in a change in reaction to tuberculin applied to the skin, just as small-pox or vaccination changed the power of reaction to vaccine of small-pox.

The Technique of the Cutaneous Tuberculin Test.—The inner surface of the forearm is washed with ether and two drops of Koch’s old tuberculin applied to the surface of the skin about 7 to 10 cm. (3 to 4 inches) apart. The tuberculin for the skin tests may be obtained in capillary tubes similar to those of small-pox vaccine. Then with a sharp instrument a small scarification is made for a control about halfway between the two drops of tuberculin. Special instruments shaped much like a small screw-driver with a platinum point may be obtained. With such an instrument the point is sterilized and applied to the skin and with slight pressure rotated until the desired scarification is obtained. Any sharp, sterile instrument may be used, however. The scarification should cover an area of the size of the head of a pin and be of such a depth that the slightest oozing of blood occurs. The scarification first described serves as a control. Similar scarifications are then made through each of the drops of tuberculin, which are then allowed to dry. No dressing is necessary.

The Cutaneous Tuberculin Reaction.—Von Pirquet describes the different reactions as follows:

Traumatic Reaction.—A few minutes after the scarification there ensues a small wheal with a surrounding areola of hyperemia. The swelling and hyperemia then disappear, and 24 hours later there is but little to be seen except slight reddening and a small crust formation. By the end of a few days to a fortnight this has also disappeared.

The Negative Reaction is similar to the traumatic reaction, and according to v. Pirquet’s observations should result in swelling and reddening not to exceed 5 mm. (¼ inch) after 24 hours. Pseudo-reactions may
result from infections, and in these slight pustule formation may occur.

Positive Reaction.—Latent Period.—The course of the reaction is for a few hours the same as the traumatic reaction. Then follows a latent period which varies from a few hours to several days. V. Pirquet designates those reactions in which the latent period covers more than 24 hours as “torpid.” As a rule, the reaction is well under way by 24 hours.

Development.—The reaction begins as an area of reddening, which soon becomes swollen and increases in size. The usual size of the swelling is about 1 cm., or a little less than ½ inch in diameter. It may be 3 cm., or about an inch and a half in diameter. In marked reactions the swelling may be accompanied by the formation of very small vesicles. The reddening of the papule may be wanting, especially in cachectic, well-advanced tuberculosis. Postule formation without secondary infection should not occur. The edges of the papule vary in distinctness. The hyperemia may extend well beyond the swelling, or slight secondary swellings may occur surrounding the papule. (Scrofulous reaction.)

Involution.—After reaching its height in about 48 hours the swelling subsides, the color changes to a darker and then to a yellowish tinge. At the end of a week the swelling has disappeared and nothing but a pigmentation remains. The reaction runs its course more quickly in young children than in older ones and may be over in three days.

General Reactions do not usually occur unless the scarification is unduly severe.

Clinical Value of the Cutaneous Test.—As in the case of the subcutaneous tuberculin test, the cutaneous reaction is a test for hypersusceptibility to tuberculin and for the presence of active tuberculous lesions. As a result of the correlation of clinical and pathologic anatomic statistics with the cutaneous test our knowledge of the frequency of tuberculosis at different ages has become much more detailed and accurate than before the introduction of the test. It has been shown by clinical findings and autopsies to be an exceedingly delicate test for tuberculin hypersusceptibility. Inasmuch as slight, healed tuberculosis as well as active processes produce such hypersusceptibility, and practically all adults have undergone at one time or another a tuberculous process, the test is given almost no weight in adults. Von Pirquet (71) gives the following figures showing the frequency of the occurrence of tuberculous processes at different ages:

<table>
<thead>
<tr>
<th>Months</th>
<th>Frequency of Tuberculosis, 988 Cases.</th>
<th>Per Cent.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–3</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>3–6</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>6–12</td>
<td></td>
<td>16</td>
</tr>
<tr>
<td>Years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–2</td>
<td></td>
<td>24</td>
</tr>
<tr>
<td>2–4</td>
<td></td>
<td>37</td>
</tr>
<tr>
<td>4–6</td>
<td></td>
<td>53</td>
</tr>
</tbody>
</table>
It will be seen that a negative reaction, with exceptions that will be mentioned, is of value in excluding tuberculous processes at all ages. A positive reaction as an indicator of active tuberculous process has a value which steadily decreases as the age of the patient increases, much as the percentages in the table. The reaction is negative in tuberculous individuals during the exanthematos stage of measles and in severe intoxications due to tuberculosis, including miliary tuberculosis. The reaction also fails during the early weeks of the infectious process. With these exceptions but few cases of tuberculosis are found which do not give a positive cutaneous test.

**Percutaneous Tuberculin Reaction (Moro)**

The tuberculin salve reaction of Moro is carried out as follows: A salve is made by triturating of equal parts of old tuberculin and lanolin. A small piece of this tuberculin salve the size of a small pea is rubbed into the skin of the epigastrium or in the pectoral region. An area of skin about 5 cm. in diameter is covered and the rubbing continued about one minute. The salve is allowed to dry for about 10 minutes. The positive test consists of the development of nodules or a rash over the area of skin affected, and sometimes is accompanied by the formation of small vesicles. These nodules vary in number and size according to the intensity of the reaction. The reaction gives about the same results as the cutaneous test but is less accurate and has but little advantage over it.

**Intracutaneous Tuberculin Reaction (Mantoux)**

Evans and Whitney (30) carry out this test as follows: The needle of a hypodermic syringe, preferably of the Luer or similar type, is inserted just beneath the superficial layers of the skin for a short distance and enough of a 1:5000 dilution of old tuberculin is injected to cause the formation of a small wheal about the size of a pinhead. A salt solution control is made a few inches away with a similar technique. An infiltration begins in a few hours and in 24 hours a red papule with a marked areola of hyperemia occurs. Involution begins soon after the height of the reaction, which occurs in about 48 hours after injection. The reaction, it is claimed, is a more delicate one than the cutaneous test.

Holmes (40) has used the intracutaneous test quantitatively; the tuberculin, injected in quantities of from 1/100 mg. to 1/100,000 mg.
dissolved in the constant amount of 1/20 c. c. of diluent (salt sol. with 0.5 per cent. of carboxic acid added), was used. The reaction was at its height in 48 hours, while the control reaction had subsided by this time. The positive reaction was characterized by an area of hyperemia 3 to 2 cm. in diameter. In this way the degree of hypersensitiveness in the different stages of the disease was ascertained. During the onset of the infection the hypersensitiveness was marked. It diminished with Improvement; fluctuated or remained stationary with nonimprovement.

Ophthalmo-tuberculin Reaction (Calmette); Conjunctival-tuberculin Reaction (Wolf-Eisner)

The conjunctival tuberculin test was performed by Wolf-Eisner (72) by instilling 1 drop of a 1 per cent. solution of old tuberculin into the conjunctival sac while the lower lid was held slightly everted for a few moments. The reaction ensues within 16 to 24 hours, and may be any one of three grades: (1) simple hyperemia, particularly of the conjunctiva of the lower lid and caruncle; (2) enlargement of the follicles of the conjunctiva; (3) severe conjunctivitis with pus formation. If no reaction occurs in 48 hours 1 drop of 2 per cent. tuberculin is dropped into the other eye. The reaction is particularly valuable in testing for tuberculosis in cattle, but owing to the severity of the reaction as it sometimes occurs it has not become popular in the diagnosis of tuberculosis in man.

Other local tuberculin reactions, including the local application of tuberculin to the nasal and urethral mucous membranes, have been devised, but have no advantages over those described.

Typhoid Fever

Typhoid-ophthalmo Reaction (Chantemesse)

Chantemesse (16) introduced the conjunctival reaction in the diagnosis of typhoid in 1907. It has not become very popular, but is undoubtedly a considerable aid in diagnosis. Recently Austrian (5) has used with satisfactory results the following technique:

Antigen Preparation.—The typhoid organisms from broth cultures of 80 strains were collected by centrifugation and by the same means washed with distilled water. The organisms were then dried in vacuo after heating to 60° C. for a half hour. The dried organisms were then ground with sodium chlorid crystals and a small amount of water added and the mixture heated to 60° C. on three successive days. The fluid was then poured into 10 times its volume of absolute alcohol and the precipitate which formed collected and dried. Ten mg. of this typhoid protein added to 1 c. c. of distilled water was used as an antigen.

Technique of the Test.—The lower lid is slightly everted and one drop
of the antigen instilled into the conjunctival sac. The reaction occurs in from 1 to 5 hours after instillation. There is a hyperemia of the conjunctiva and caruncle which deepens to a purple hue. After reaching its height in from 10 to 20 hours involution occurs. It may take as long as 240 hours for the reaction to run its course. The reaction is specific with the exception that group reactions (paratyphoid, dysentery) occur. Its advantage depends upon the fact that a positive reaction occurs earlier in typhoid than the Widal reaction, and the technique of the test is easy.

**Gonococcal Infections**

**Reactions to Subcutaneous Inoculations of Vaccines.**—In 1908 Irons (41) described reactions following the subcutaneous inoculation of gonococcal vaccine into patients suffering from gonococcal arthritis. He observed (1) a local reaction at the site of injection, consisting of redness and swelling appearing a few hours after the injection and lasting 24 to 48 hours; (2) a focal reaction in the affected joints consisting of increased pain and tenderness; (3) a general reaction with rise in temperature and general malaise. Irons considered such reactions as of a limited diagnostic value in arthritis. In 1909 Bruck described similar phenomena in gonococcal epididymitis following subcutaneous inoculations of gonococcus vaccine. The reaction has been employed also by Reiter and others in the diagnosis of pelvic infections in women.

In cases of suspected gonococcal arthritis a positive reaction is of value in distinguishing the gonococcal from other forms of arthritis. In performing the test the size of the dose of vaccine necessary to give a reaction will vary with the vaccine as well as with the condition of the patient. With the ordinary polyvalent vaccines a dose of 100- to 500,000,000 is suggested. If the vaccine is prepared from young, recently isolated cultures smaller doses are advisable. The hypersusceptibility of patients seems to vary in the same way as is noted in tuberculosis.

**Cutaneous and Intracutaneous Gonococcal Reactions.**—Cutaneous reactions have been obtained by introducing gonococcal vaccines into the skin by the method of v. Pirquet. London, Reiter, and others have used intracutaneous inoculations and have obtained specific reactions. Glycerin extracts of gonococcus protein have been used for inoculation by the method of v. Pirquet (Irons) by which reactions apparently specific were obtained.

The cutaneous hypersusceptibility of persons infected with the gonococcus seems to vary in different individuals, and in the same individual from time to time. The difference in reaction of different extracts has been ascribed to the varying degree of autolysis of the gonococcus protein during their preparation. For these reasons the practical value of cutaneous gonococcal reactions is limited.
Syphilis

The Leutin Reaction (Cutaneous Test for Syphilis)

With an idea analogous to that of Wassermann, who substituted extract of spirochete-containing organs for pure cultures in the Bordet-Gengou phenomenon, a number of experimenters attempted to devise a cutaneous test such as the tuberculin skin test by substituting extract of spirochete-containing tissues for the pure antigen. These attempts, unlike that of Wassermann, were unsatisfactory, and it was not until Noguchi was able to obtain quantities of the spirochaeta pallida in pure culture that a successful cutaneous reaction was obtained. This was accomplished in 1911, and in the same year Noguchi (67) described his cutaneous test.

Preparation of Antigen.—Noguchi prepared the antigen by growing pure cultures of spirochaeta pallida in ascitic fluid containing placental tissue and in ascites-agar containing placenta. The agar in which the spirochetes were abundant was freed from tissue and ground in a sterile mortar, enough fluid media being added to form from this paste a liquid emulsion. The antigen was then heated for a half hour in a water bath at 60° C. and .5 per cent. tricresol added. The antigen was tested for sterility on culture media and by injection into the testes of rabbits. Noguchi speaks of this antigen as leutin. A control was prepared from the media alone in a similar manner. With this antigen Noguchi was able to produce a cutaneous reaction in animals with experimental syphilis and the test was then applied to patients.

Technique of the Test.—The upper arm is washed with alcoholic sublimate solution and 0.07 c. c. (one drop) of a 1:1 dilution in salt solution of the antigen injected intracutaneously. That is, a fine needle is inserted just beneath the epidermis and advanced for a short distance parallel with the surface of the skin, after which the fluid is injected. In a corresponding place on the other arm a similar injection is made with the control preparation.

Negative Reactions.—Usually in non-syphilitic individuals there appears in about 24 hours at the site of inoculation a small rosy areola with, at times, slight swelling from 24 to 48 hours after injection. The reaction begins to undergo involution within 72 hours and disappears.

Positive Reactions.—Noguchi (55) describes three positive reactions:

Papular Form.—The papule develops in from 24 to 48 hours and reaches in this time a diameter of from 7 to .10 mm. It continues to increase in size, and becomes surrounded by a red areola. The stage of evolution lasts about four or five days, after which involution begins and usually disappears in about two weeks. During involution the color changes from a bright to a bluish-red and then fades.

Pustular Form.—This begins in the same way as the papular form, and after continuing four or five days the papule become edematous, and
<table>
<thead>
<tr>
<th></th>
<th>Primary Syphilis</th>
<th>Secondary Syphilis</th>
<th>Tertiary Syphilis</th>
<th>Congenital Syphilis</th>
<th>Cerebrospinal Syphilis, Symptoms Present</th>
<th>Latent Syphilis</th>
<th>Controls, Non-syphilitic Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Symptoms Present</td>
<td>Symptoms Present</td>
<td>Symptoms Absent</td>
<td>Symptoms Present</td>
<td>Under one Year</td>
<td>Late Cases</td>
<td></td>
</tr>
<tr>
<td>Luetin test..........</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>No treatment........</td>
<td>-</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slight mercurial treatment</td>
<td>1</td>
<td>12</td>
<td>2</td>
<td>25</td>
<td>12</td>
<td>-</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regular mercurial treatment</td>
<td></td>
<td>14</td>
<td>3</td>
<td>31</td>
<td>29</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>606 and mercury.....</td>
<td>1</td>
<td>-</td>
<td>42</td>
<td>12</td>
<td>22</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total...</td>
<td>1</td>
<td>25</td>
<td>3</td>
<td>25</td>
<td>56</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>43</td>
<td>0</td>
<td>51</td>
<td>4</td>
<td>6</td>
<td>21</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5</td>
<td>24</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>250</td>
</tr>
</tbody>
</table>
vesicles appear. A softening of the papule then takes place and in 24 hours the papule becomes a vesicle and then a pustule. The pustule usually ruptures and becomes covered with a crust and surrounded by an indurated inflammatory zone. After a few days the crust falls off, leaving an indurated nodule which remains from a few weeks to a number of months. This reaction is found in tertiary or late hereditary syphilis, or secondary syphilis, when treated with salvarsan.

**Torpid Form.**—This unusual reaction acts as does a negative one, and then, often ten days or longer, develops into a pustular form. This type is found in cerebrospinal, primary, secondary, or congenital syphilis.

In most luetin tests only a slight febrile reaction occurs, but in some cases malaise, anorexia, and diarrhea were noted. In some cases moderate reactions occur at the control inoculations as well as at the site of the test. The test inoculation, however, gives a more pronounced reaction.

**Diagnostic Value of the Reaction.**—The reaction is positive in almost all cases irrespective of syphilis if obtained before, during or shortly after the administration of iodids, as shown by Sherrick (77). Noguchi's table (page 161) shows the number of reactions in the different forms of syphilis.

It will be seen from this table that the highest percentage of positive reactions is obtained in the tertiary stage of syphilis, and in latent syphilis. In a study of a variety of cases Wolfsohn (89) and more recently Hanes (38) came to similar conclusions. He found in parasyphilitics the reaction might be delayed for from 9 to 30 days. He also found the highest number of reactions on the control site highest in tertiary syphilis. It will be seen that the greatest number of positive reactions occur in cases where the Wassermann reaction gives the lowest number of positive tests, thus making the luetin test of value as an adjunct to the Wassermann reaction.

**The Result of Treatment on the Luetin Test.**—Unlike the Wassermann test, the luetin test is often brought out by treatment where it is negative before treatment and is afterward less readily influenced by treatment. Salvarsan plus mercurials produces the most marked results according to Noguchi in bringing about positive tests and in causing their disappearance afterward. Noguchi considers the presence of a positive luetin test in the cases with negative Wassermann reactions of especial value as an indication for continuing treatment. Allergic reactions have been described in tricophyton infections by Amberg (3) and in leprosy by Duval and Gurd (29), but have not been widely used in diagnosis.

**REFERENCES**

REFERENCES

8. Blaschko and Swift. Quoted by Pearce.
15. ——. Loc. cit.
20. ——. Quoted by Noguchi.
36. Hamman and Wolman. Tuberculin in Diagnosis and Treatment.
44. ——. Münch. med. Woch., 1911, No. 25.
68. ——. Serum Diag. Suph., Phil., 1912.
72. ——. Quoted.
75. Schmorl. Quoted by Jellinghaus and Losse.
83. Vincent and Bellot. Quoted by Paltauf.
87. ——, Neisser, and Bruck. Deutsch. med. Woch., 1906, xxxii, 745.
CHAPTER IV

FOCAL INFECTION IN RELATION TO SYSTEMIC DISEASE

FRANK BILLINGS

The principle of the development of a systemic or localized disease from a previously existing infectious focus is a long-established fact. Rheumatic fever, endocarditis, generalized tuberculosis, gonorrheal arthritis, and septicopyemia are familiar examples. Not only acute, but chronic systemic disease, including cardiovascular and visceral degenerations, may be caused by a chronic focal infection. Chronic focal infection may exist for a long period without apparent harmful result; the defenses of the body probably prevent general infection.

It is also true that an insidious slow systemic intoxication may occur from a focal infection which is finally recognized because of disturbed function of various organs. Myocardial degeneration, chronic nephritis, and arterial fibrosis are the most common expression of the slow, insidious intoxication. Of course, other factors—inheritance, a bad personal hygiene, food and drink abuses, occupation, etc.—may play the more important part in these degenerative processes; but exclusive of these recognized etiologic factors, chronic focal infection may be the cause of cardiovascular and kidney and other disease. The focal infection may disappear spontaneously and coincidentally the evolution of the systemic disease may cease, leaving the patient more or less an invalid, or entire recovery may occur. This is witnessed in individuals suffering from chronic arthritis, myocarditis, and even in moderate grades of nephritic disease.

SITE OF THE FOCUS

The focus, acute or chronic, may occur anywhere in the body. Usually the focus is located in the head, probably because the mouth and air passages are so frequently exposed to infection. Bacteria-laden air, insanitary dwellings, faulty individual mouth hygiene, etc., play an important part. In childhood the lymphoid tissue of the nose and throat may be excessive and apparently affords a favorable soil for infection. The faucal tonsil
and adenoid overgrowth in the nasopharynx are the frequent seats of infection. Obstructed infected crypts of the tonsil due to chronic tonsillitis or to the sealing scar of tonsillotomy are the most common focal source of many systemic diseases. Dental alveolar infection, especially chronic abscess, curiously often unperceived by the patient, is a frequent source of general debility, chronic arthritis, etc. Modern dentistry, characterized by wonderful technical skill in the use of gold crowns, bridgework, etc., is sometimes the cause of the alveolar focus of infection.

Chronic infection of the various sinuses of the head, especially if undrained mucopus exists, may cause systemic disease.

Infection may pass from the throat and sinuses along other mucous tracts and involve the eyes and also the middle ear and mastoid cells, or it may pass through the lymphatics to the meninges or to the lymph glands of the neck. The lymph glands so infected may form additional foci of danger to systemic disease. The genito-urinary infections are frequent sources of general disease. Gonorrheal septicopyemia and arthritis are examples. Urinary stasis from prostatic enlargement, stenosis of ureters, foreign bodies, etc., is usually associated with colon, streptococcus, bacillus pyocyaneus, or other bacterial infection, and may be the cause of systemic disease.

Cholecystitis and cholangitis may cause septicemia, and when chronic may cause degenerative changes in the heart, blood vessels, and kidneys. Chronic appendicitis may be a cause of local distress and a danger to life through abscess formation with rupture and resulting septic peritonitis. Quite as dangerous to health and life may be the resulting degenerative changes of myocardium, arteries, kidneys, and other organs of surgically neglected chronic appendicitis. Local septic foci of the submucous and subcutaneous tissues anywhere may cause systemic disease. Septic venous thrombi due to infection of contiguous tissues are sources of septicemia.

The intestinal tract may be the source of invasion of bacteria, as in typhoid fever, cholera, dysentery, etc., as water- or food-born infections. These general diseases do not fall under the principles of this article.

Much less has been written of the chronic, local, and systemic disease due to the intestinal bacteria. Probably under abnormal anatomical conditions of the tract, with stasis of intestinal contents and sluggish blood circulation, ordinarily innocent bacteria (colon, streptococcus intestinalis, etc.), may acquire pathogenic virulent properties with resulting local and systemic disturbances of various organs. Unusual intestinal bacteria (B. aerogenes capsulatus, B. proteus vulgaris, streptococcus viridans, and streptococcus pyogenes, etc.) may have an etiologic relation to pernicious anemia, chronic arthritis, cardiovascular and visceral degenerations. Careful investigation, fully controlled to avoid erroneous conclusions, must be made to settle the various hypotheses.
SYSTEMIC DISEASES OF FOCAL ORIGIN

The systemic diseases which may be focal in origin may be divided into the acute and chronic forms.

Of the acute diseases rheumatic fever, malignant endocarditis, simple endocarditis, streptococcemia, staphylococcemia, gonococcal septicopyemia, and arthritis are familiar typical examples. The chronic systemic diseases of focal origin may be chronic arthritis (streptococcus, gonococcus, etc.), myositis, neuritis, myocarditis, nephritis, arteriocapillary fibrosis, and degenerative processes in various viscera.

The focus of systemic infection may apparently give rise in one individual to an acute process, and in another to a chronic disease. This is especially true of the acute and chronic forms of arthritis, myositis, and endocarditis and appears to be due to the modification which the streptococci, the usual cause, may undergo in known mutation of cultural characteristics and pathogenicity in varying culture media and serial animal inoculations. (Rosenow, 5.) Clinical observations and coincident bacterial experimentation apparently prove this statement. Strains of streptococci (streptococcus viridans, streptococcus mucosus, streptococcus hemolyticus, streptococcus rheumaticus, etc.) have been obtained by cultural methods from infected crypts and abscesses of tonsils, dental alveoli, and other foci; from the exudate of sinusitis; from joint exudates in acute and chronic arthritis; from excised muscle in chronic myositis; from the blood in malignant and simple endocarditis; from the fibroid nodes upon the sheaths of tendons and aponeurosis of muscles in arthritic patients, and finally from enlarged lymph nodes near the infected joints. As an example the streptococcus obtained from the joint exudate of rheumatic fever, by the special methods employed (Rosenow, 6) has been so changed in its cultural characteristics and pathogenicity that in the experimental animals myositis, arthritis, endocarditis, or pneumonia, etc., resulted coincidentally with the phases of mutation of the micro-organism.

It seems rational to make the deduction that mutation of specific pathogenicity takes place in the streptococcus-pneumococcus group in the focus of infection. Acute streptococcus tonsillitis may occur immediately before or during rheumatic fever. Often there is a history of one or more attacks of "sore throat" in previous weeks, months, or years. The same story is of common occurrence in the more chronic arthritic, muscular, and endocardial diseases. The streptococci in the latent focus have changed in specific pathogenic character because of some biochemical qualities of the tissue in which they lie. Probably the local blood supply and the oxygen content of the locally infected tissue play an important part in the mutation.

With the defenses of the body diminished by overwork, dissipation,
exposure to cold, insufficient or improper food; by unhygienic surroundings; by injuries from previous disease (valvular scar), or trauma (joint or muscle) the individual may suffer from acute or chronic arthritis, myositis, or malignant or simple endocarditis or pneumonia, dependent upon the phase of mutation in pathogenicity of the specific strain of the streptococcus-pneumococcus group in the local focus.

The Relation of the Suspected Focus to the Systemic Disease.—The relation of a suspected focus to the systemic disease seems to be proved in many instances by several factors. The removal of the infected focus by surgical or other means is sometimes followed by rapid recovery from the systemic disease. Many observers have noted the great improvement in the general health by tonsillectomy, removal of post-nasal adenoids, drainage of a chronically infected gall-bladder, appendectomy in chronic appendicitis, and removal of carious teeth and relief from alveolar dental infection. One must recognize the improvement in the ability to breathe when obstruction of the air passages is relieved by tonsillectomy and removal of adenoids; a better digestive power and consequent improved nutrition by correction of dental faults, relief of cholecystitis and appendicitis, but, admitting this, it seems obvious that relief from continued systemic infection is the chief reason for the general improvement.

In focal infection of the jaws, tonsils, sinuses, etc., cultures obtained from the abscesses, exudates, and removed tissue usually yield pathogenic strains of the streptococcus group, streptococcus hemolyticus, and streptococcus viridans predominating. Subcultural experiments and animal inoculation with these strains afford the characteristic results noted above. Inoculation of patients with autogenous antigens made from cultures obtained from the infectious foci often produce local and general reactions and increase the opsonic and phagocytic index. Patients recover entirely or the further progress of the systemic disease ceases with the removal of the focus and a subsequent management which establishes an immunity to the infectious micro-organism still in the tissues. Finally success does not readily follow any treatment or management which does not include the complete removal of the focal infection.

THE PATHOLOGY OF CHRONIC SYSTEMIC INFECTION OF FOCAL ORIGIN

The streptococci in the focus of infection apparently attain specific pathogenic qualities (see above) with affinity for joint tissues, kidneys, muscles, including myocardium, gastro-intestinal mucosa, gall-bladder, endocardium, lung, etc., respectively.

The specific streptococci pass through the blood stream and lodge in the arterioles and capillaries of the organ or tissues as embolic masses.
Small hemorrhages result in heart valves, muscles, mucosa of stomach and gall-bladder, kidney, etc. (Rosenow, 7.) As a result of the embolism and hemorrhages, characteristic changes occur in the infected tissues and elsewhere in the body. Rosenow (8) has shown in experimental animals hemorrhages, subsequent ulceration, and characteristic massive vegetations with contained thrombi of the heart valves; hemorrhages and subsequent leukocytic infiltration and degeneration of voluntary muscles and myocardium; hemorrhage into and subsequent ulceration of the mucous membrane of the stomach and intestine; hemorrhage into and subsequent infection of the gall-bladder; hemorrhage of the glomeruli of the kidneys with hematuria, cylindruria, albuminuria, etc. Similar pathological processes have been obtained in the clinical and pathologic studies of patients suffering from malignant endocarditis, myositis, cholecystitis, ulcer of stomach, hemorrhagic nephritis, etc. Cultures of the specific coci have been obtained from the lesions named in both animals and patients.

Additional pathologic changes occur which are characteristic of the organ primarily or chiefly involved. The massive vegetations and contained thrombi serve as a rich culture medium for the specific streptococcus (streptococcus viridans) in malignant endocarditis, with consequent constancy of the streptococceum. In the chronic type of the disease the defenses of the body (antibodies) apparently become exhausted; the infectious organism becomes immunized against the host (Welch). The streptococci are also disseminated throughout the body by means of the detached particles of vegetations and thrombi, which lodge as emboli in all the organs and tissues. This generalized embolism may produce constitutional disturbance and various local phenomena (petechia of skin, hematuria, splenomegaly with splenic tenderness, hemiplegia, etc.). In one patient included in a clinical report by Billings (1) local endarteritis with small aneurysm resulted. An aneurysm of one of the branches of the gluteal artery ruptured into the muscle and an abscess formed. From the abscess the same type of streptococcus was obtained as that found in the circulating blood.

The infected voluntary muscle groups and their tendons and aponeuroses are tender, painful and contracted in the acute stage. In the chronic stage, painless when at rest, they are shortened by contraction from interstitial degeneration and thickening due to the infection, local anemia, and non-use.

The small submucous gastric embolic hemorrhage is followed by anemic necrosis and subsequent digestion of dead tissues. The acute ulcer may bleed and imperil life or a typical chronic peptic ulcer may be the final result.

In the gall-bladder the embolic focus and hemorrhage are usually located at the base, the situation of the terminal blood vessels. The rupture of this submucous focus into the gall-bladder may cause cholecystitis and
FOCAL INFECTION AND SYSTEMIC DISEASE

gall-stones also may form. Hematogenous embolic infection of the soft tissues of the joints occurs in experimental inoculation of animals. Similar embolic hemorrhages occur in the capsule, the synovial sac and fringes and bones in man and animals.

The changes which occur in the cartilage, bones, and other joint structures in chronic deforming arthritis are illuminated by the experiments of Oxhausen (4). The simple aseptic necrosis of bone and cartilage resembling the morbid anatomy of atrophic and also hypertrophic types of arthritis deformans was produced by the ligation of some of the arteries supplying the joint, with resulting anemia of joint structures. Injuries of joints resulting in diminished blood supply have been known to produce a like morbid anatomy of the joint. It may be that the anemia plus the toxins of embolic joint infection will explain the hitherto unknown metabolic changes of chronic arthritis. The general malnutrition and anemia so commonly present in this class of patients would be an additional factor.

RESULTS OF SECONDARY FOCI OF INFECTION

The secondary foci in the various organs and tissues are capable in some instances of intensifying the systemic disease. Mention has been made of the growth of bacteria in the thrombi-containing vegetations on the heart valves in malignant endocarditis. The condition furnishes a constant bacterial multiplication which is added to the blood stream. The usual infectious organism in this type of endocarditis is the streptococcus viridans. The peculiarity of this organism is that it has only moderate virulence as compared with many other strains of streptococci. One of its peculiarities is that it requires a high oxygen tension for its growth, and this it finds as a surface growth in focal infection and in the blood stream in malignant endocarditis. Probably it is this peculiarity of this type of streptococcus, and the fact that the thrombic vegetations which it produces on the heart valves act as a good cultural medium for it, that make this disease so fatal. It finds on the heart valves a good secondary focus where it may grow, and it finds a rich oxygen content in the blood stream.

Infected lymph nodes proximal to the focus of infection may become secondary foci. General tuberculosis, acute and deforming rheumatism, endocarditis, simple and malignant, and other systemic disease may develop from the secondary foci.

The embolic foci of the systemic disease are found in muscles and other tissues, and have been shown by Jackson (3) to occur in the blood vessels of the bones and of the tissues of joints. The fact that the infection occurs in an embolic form, including many blood vessels, and thereby reducing the blood supply of the infected organs, explains many of the peculiarities of the chronic types of myositis and arthritis. The injury
TREATMENT

to the blood vessels partially deprives tissues of blood, and thereby interferes with their nutrition and oxygen supply. The types of streptococci which infect muscles and cause chronic arthritis have also a low virulence. They grow best in a low oxygen tension. The fact that the embolic process deprives the tissues of blood and lowers the oxygen content furnishes the best possible conditions for continued viability and probably also for multiplication of the infectious micro-organism. This peculiarity of the pathology of the chronic types of myositis and arthritis also explains the progressive morbid anatomy so peculiar to these diseases. The metabolic changes which occur in the muscles and also in the bones and cartilages of the joints seem to depend upon the deprivation of the structures of those elements necessary for their general nutrition. Therefore, in the treatment which will restore the condition it is necessary that the nutritional side of the tissue be considered, attempts being made to restore circulation and full oxygen content to the tissue before the infectious micro-organism can be expelled and the morbid anatomical changes stopped. It explains the reasons for the improvement of patients who are managed along the lines of general support, including the improvement of the general nutrition of the body by good food, plenty of oxygen in the form of pure air, passive and active exercise commenced mildly and gradually increased, and all other measures which tend to build up the general health. One can also understand why patients so managed without a removal of the primary focus of infection may relapse because of reinfection. It explains why these patients are made definitely worse by all exhausting and depressing measures such as an insufficient diet with low protein content; exhausting warm or hot baths and mental and physical fatigue.

FOCAL INFECTION AND ANAPHYLAXIS

The principles of anaphylaxis are especially and exhaustively explained elsewhere. The subject is mentioned here only to emphasize the fact that the body may be sensitized by the absorption of a protein substance from a focus of infection. This may result in periodic evidence of anaphylaxis in the form of urticaria and other skin lesions, asthma, etc.

TREATMENT

PROPHYLAXIS

Focal infection is most commonly situated in the head, but may be located in any organ or tissue. The mouth and air passages are constantly exposed to infectious bacteria, especially in individuals who live in densely
FOCAL INFECTION AND SYSTEMIC DISEASE

populated centers. Insanitary environment usually cannot be controlled. When possible this should be commanded. Individual hygiene should be enforced by municipal, county, and state health officers. This would be feasible in all public school children. The enforcement of a personal hygiene by public officers would educate and impress parents and other individuals with its importance. Enlarged or infected faucial tonsils, adenoid tissue overgrowth, and carious teeth are a menace to health and life. Tonsillectomy, thoroughly performed, may save the individual, especially a child, from local infection in the form of tonsillitis, peritonsillitis, diphtheria, etc., and also from consequent rheumatic fever, endocarditis, tuberculous lymphadenitis of the neck and mediastinum, hemorrhagic nephritis, acute and chronic myositis, chronic deforming arthritis, etc. Tonsillectomy should not be needlessly practiced, but when there is evidence that the tonsils are infected or enlarged by chronic disease they should be thoroughly enucleated to prevent further local and possible systemic disease. The function of the normal tonsil is not known. Its removal has not been followed by any recognizable local or constitutional disturbance. An infected or abnormal tonsil is a harmful organ and should be wholly removed. Partial removal (tonsillotomy) is a temporizing, dangerous measure. The remaining crypts, sealed over by the operation scar, afford a condition as bad or worse than the original tonsil.

Excessive adenoid tissue of the nose and pharynx prevents free drainage and obstructs the air passages. In addition to the local effects, the danger of middle ear, mastoid and lymph gland infection, and possible systemic disease should indicate prompt operative correction.

Carious teeth are an inexcusable evidence of faulty personal cleanliness in those who are otherwise healthy. Constitutional conditions may, of course, be a cause of, or at any rate be associated with, caries and other diseases of the gums, teeth, and jaws. Caries of the teeth may lead to septic disease of the gums, to alveolar abscesses, etc. Systemic infection may result. In children and others proper dentistry should be instituted to prevent focal disease, as well as the possible subsequent chronic arthritis, furunculosis, general debility, etc. Modern dentistry has technical faults. The use of metal crowns upon teeth with infected pulp results, in many instances, in the establishment of mechanical dams over infectious foci. In my opinion the general use of crowns and other dental measures which are likely to shut in infectious foci should be abandoned.

Cholecystitis, especially if chronic, is a recognized cause of systemic disease, especially visceral degenerations. Myocardial degeneration is frequently associated with it. Improvement of the heart condition is often noted after cholecystotomy and drainage. Surgical treatment of cholecystitis and cholangitis is indicated, not only to relieve the local disease, but it is quite as important, to prevent systemic slow intoxication and consequent myocardial and other visceral degeneration. Surgically neglected,
appendicitis may be a local menace, may disturb the organs of digestion and in addition may cause systemic chronic intoxication and cardiovascular, kidney, and other organic degenerative changes. Neglected gonorrheal foci, located in the deep urethra, mucous glands of the prostate and in the seminal vesicles are dangerous in the dissemination of the disease in sexual intercourse and also of systemic infection of the host in the form of arthritis, tenosynovitis, septicemia with malignant endocarditis, etc.

Septic conditions of the urinary tract, especially those due to defective drainage from pelvic disease of women and to morbid anatomical changes of the prostate, bladder, ureters, and kidneys, should receive appropriate surgical treatment and medical management to relieve local conditions and to prevent additional serious systemic disease.

Finally prevention of systemic disease from a focal infection should be promoted by all of the means which are known to maintain the natural defenses of the body, namely: pure air, simple good food, avoidance of over-fatigue and exposure to extreme changes of temperature, especially that which lowers the temperature of the body for a relatively long period.

**Method of Treatment**

The patient who suffers from acute or chronic arthritis, endocarditis, myositis, hemorrhagic and chronic nephritis, etc., should have repeated thorough physical examinations. Careful search should be made to locate the infectious focus. This is not always evident or easily found. That it is most frequently present in the faucial tonsil should not lead to hasty tonsillectomy in all patients. Advantage should be taken of the Roentgen ray; of transillumination, and of the aid of throat and nose specialists in examination of the head. A complete history, careful physical exploration of the abdomen, test meals; fluoroscopic bismuth tests; microscopic chemical and bacterial cultures of stools may be necessary to recognize chronic foci in gall-bladder, appendix vermiciformis, or elsewhere in the gastro-intestinal tract and of intestinal stasis with abnormal and pathogenic intestinal flora. Thorough investigations should be made of the genito-urinary tract by pelvic exploration and urine examination, chemical, microscopic, and, if necessary, by bacterial cultures. Massage of the prostate and seminal vesicles may yield the gonococcus and afford an immediate recognition of the cause and nature of the systemic disease. A denial of an acquired gonorrhea or the confession of an infection many years before should not excuse this examination in every male patient who suffers from arthritis.

Occasionally one will find the focal infection in an unusual place. A suppurating toe from an ingrowing nail has been the source of rheumatic fever with pancarditis in one patient, and of chronic deforming arthritis in another. Specific streptococci were obtained in pure culture from the pus under the toe nail from both patients.
Removal of the Focus of Infection.—When ascertained, the focus of infection should be eradicated by the necessary surgical aid or other means, which have been fully explained under prophylactic treatment. Secondary foci in the form of enlarged lymph nodes should also be surgically removed if there is a probability that they may continue to cause general infection as secondary foci.

In acute conditions like rheumatic fever, malignant endocarditis, and the like it may be hazardous to attempt to remove the primary focus. It is questionable whether recognizably infected tonsils should be removed during the height of rheumatic fever. Inasmuch as many individuals are apt to have repeated attacks of acute rheumatism, the apparent focal cause (usually infected faucial tonsils) should be removed in the interval between attacks. In chronic types of infectious endocarditis it is wise to remove a recognized primary focus.

Bacteriologic Examination.—Bacterial cultures should be made of the tissue and exudates of the focus of infection, of effusions of joints and other serous sacs, of excised pieces of muscle, lymph nodes, fibrous nodes, etc., to arrive at a reasonable bacteriologic diagnosis. In endocarditis a blood culture is necessary to learn the nature of the bacterial infection. A well-trained bacteriologist armed with an adequate laboratory equipment will be able to make successful cultures if he adopt the technique now well known to all experienced laboratory workers. Bacterial cultures enable one to recognize the type of infection, and also furnish an autogenous bacterial vaccine if it is desirable to use that form of treatment. If conditions make it impossible to have bacterial study made of the tissues and exudates found in a focus of infection the patient may be cared for along general lines of management modified to meet the type of systemic disease, as is mentioned in the preceding paragraphs on treatment. In any event if there is a sign of a focus of infection it must be removed.

General Management.—The management of the patient after the removal of the focus of infection will, of course, depend upon the character of the systemic disease from which he suffers. Details of this management for each systemic disease cannot be suggested in an article of this kind. An attempt is made here to establish knowledge of the principles involved in the subject. The patient who suffers from malignant endocarditis must be treated in general as indicated in the literature which may be commanded. So, too, acute rheumatic fever, chronic deforming arthritis, gonorrheal arthritis, etc., must be managed as indicated in the numerous articles written upon those subjects.

Vaccines and Serum Treatment.—Vaccines have been used as specific methods of treatment in many of the systemic diseases due to focal infection. Autogenous vaccine has been extensively used in malignant endocarditis due to the streptococcus viridans. Improvement by such vaccine has been reported, but it is the experience of the author that the use of
vaccine in patients suffering from malignant streptococcal endocarditis is without benefit. Indeed, it seems that in some patients so treated by large doses, 500,000,000 to 1,000,000,000 of the autogenous vaccine, distinct harm has resulted. Possibly small doses may increase the defenses of the body of the patient in this disease, but for the reasons stated in the paragraph on the pathology of the condition it is not likely that any remedy now known will affect the large vegetations upon the heart valves and produce antibodies in the blood stream which will affect to any appreciable degree the life of the infectious organism.

In acute rheumatic fever autogenous vaccine has not been sufficiently tried to enable one to make a definite statement concerning the value of the treatment. Stock vaccines so used have not produced good effects with regularity or uniformity, and the good results which have been reported are just as likely to have resulted from other influences, inasmuch as the natural clinical course is often changed by non-specific measures. The peculiarity of rheumatic fever in running a definite and limited course, as was shown by the elder Flint, makes all deductions concerning the use of remedies, whether drug or specific vaccines, a question which requires proof by the study of a large number of cases, properly controlled. The disease is not usually dangerous to life, so that the proof of the value of a "specific" remedy by the fact that 75 per cent, or more of patients recover is begging the question. The fact that endocarditis, with resulting crippled heart valves, occurs in so many young patients who suffer from rheumatic fever is the important thing which should encourage one to seek for a method in the treatment of rheumatism which is specific. Until that time comes the wisest thing to do is to use prophylactic measures to prevent the disease and to follow well-known and established drug treatment and rational management.

The use of vaccines in chronic deforming arthritis and myositis has been practiced extensively. In the study which has been made of the effects of autogenous vaccines by the author and those working with him it may be said that vaccine made from the tissues and exudates of the focus of infection, and also that made from the micro-organisms obtained from the muscles and joint exudates of the patient, have seemed to possess some value. This seemed to be shown by more or less local and general reaction in some of the patients. The local reaction is shown in the form of slight redness and tenderness of the skin at the point of the subcutaneous inoculation, and the general reaction by a sense of general discomfort, sore and aching muscles and joints, and sometimes a moderate rise of temperature on the day following the injections; also by an improvement of the opsonic and the phagocytic index of the patient's blood as shown by examination before and after the injections. Large doses of the vaccine have not proved more efficacious than moderate ones. Consequently the large dose of 1,000,000-000 or more which was formerly given is now reduced to 100,000,000 as an
average. It is the opinion of the author that while autogenous vaccines may be specific to some degree in the treatment of chronic arthritis and myositis the good result obtained in the management of these patients is due more largely to the improvement of the general health by the measures of general and individual hygiene which have been mentioned. Failure will occur in the management of this class of patients if reliance is placed wholly upon vaccines.

Serum.—In chronic arthritis and myositis a polyvalent streptococcus horse serum has been used. The serum was prepared by immunizing two horses with approximately thirty strains of streptococci of various types obtained from patients suffering from chronic arthritis and chronic myositis. The aged, refined, and heated serum was used coincidently with the autogenous vaccines. Under this management the defenses of the body seemed to improve more rapidly than with vaccines alone, as was manifested in a higher curve of both the opsonic and phagocytic index. Unfortunately the serum sensitized every individual upon whom it was used, and the use of the serum subsequent to the second or third dose produced more or less serum reaction (anaphylaxis). Usually this consisted of skin eruption—erythema and urticaria with intense itching—but in three patients the reaction amounted to a severe degree of anaphylactic shock and an alarming condition. Consequently the use of the serum was abandoned, as it was believed that the removal of the focus of infection followed by the general hygienic management mentioned, and the use of the less dangerous autogenous vaccines would be successful, without the serum. Autogenous colon vaccine has an unquestionable value in colon infections of the urinary tract. (Billings, 2.) But to be successful there must be no stasis of urine in the tract. If there exist any morbid anatomical conditions (stricture of urethra, prostatic enlargement, stenosis of ureters from any cause, calculus or other foreign body in the tract, etc.) the infectious bacteria in the urine will persist until the cause of the stasis is surgically removed, and then vaccines will aid very much in rendering the urine sterile. If residual urine is associated with colon infection, daily bladder irrigation and the use of vaccines may give good results. However, as long as the cause of residual urine persists reinfection is apt to occur.

The use of what may be called polyvalent bacterial filtrates in any of the focal or systemic diseases mentioned in the subject of this paper is not justified by scientific experiments, rational deduction, or clinical results.

The use of vaccines and sera in gonococcal infections, asthma, furunculosis, and other diseases, focal and systemic, is discussed in the chapters relating to those subjects.

REFERENCES

5. Rosenow, E. C. Transmutation Within the Streptococcus-Pneumococcus Group, Jour. of Inf. Dis., Jan., 1914, xiv, 1.
8. ———. The Production of Ulcer of the Stomach by Injections of Streptococci, J. A. M. A., Nov. 29, 1913, lxi, 1942.

**Bibliography Not Directly Quoted**

Billings, Frank. Chronic Focal Infections and Their Etiologic Relations to Arthritis and Nephritis, Arch. Int. Med., Apr., 1912, ix, 484.
———. Chronic Focal Infection as a Causative Factor in Chronic Arthritis, J. A. M. A., Sept. 13, 1913, lxi, 819.
Davis, David J. Chronic Streptococcus Arthritis, J. A. M. A., Sept. 6, 1913, lxi, 819.
CHAPTER V

THE PROPHYLAXIS OF TYPHOID FEVER BY MEANS OF VACCINES

FREDERICK F. RUSSELL

Historical.—The history of the subject is closely identified with the development of our knowledge of immunity. As all early theories led to no clear-cut explanation of the well-recognized condition of immunity which almost invariably follows an attack of typhoid fever they have been either abandoned or profoundly modified.

The fundamental fact on which the entire procedure rests is that one attack, with rare exceptions, protects the individual for life. Osler says, "Of 2,000 cases of enteric fever at the Hamburg General Hospital only 14 were affected twice, and but one person 3 times. In 500 of our own cases, in which special inquiry was made as to a previous attack, it was found to have occurred in 11 or 2.2 per cent."

The earliest attempt to produce immunity artificially against typhoid fever was made as long ago as 1886 by Simmons and Frankel (13) some six years after the bacillus typhosus had been discovered by Eberth. They used small laboratory animals, and succeeded in increasing the resistance to lethal doses of bacteria. Later their work was confirmed and extended by Beunmer and Piper, and in 1888 by Chantemesse, Widal, Sanarelli (13), and others.

Little or nothing came of these early experiments, largely because of the impracticability of using living bacteria on man, and because there was then no satisfactory method of determining the existence of immunity in human beings, or of estimating its degree by examination of the blood serum.

In 1892 Brieger, Kitasato, and Wassermann (3) found that the use of living bacteria was unnecessary, and that a high degree of immunity could be produced by killed cultures. In 1893 and 1894 R. Pfeiffer reported his investigations on the nature of the immunity in typhoid fever and cholera, and elaborated a test for the presence of the bacteriolytic protective bodies in the blood which has since become classic under the name of the Pfeiffer phenomenon.

In 1896 Gruber and Widal discovered the presence of agglutinins in
the blood, and as a result our knowledge of changes in the blood serum during and subsequent to typhoid fever increased rapidly.

In the latter part of this year (1896) Pfeiffer and Kolle (5), using killed cultures of the bacillus, immunized 2 men against typhoid fever, and made complete and comprehensive studies of the changes in the blood serum during the progress of immunization.

Although their report covers only 2 cases, it is most convincing because of the completeness of the investigation; for they found, even after a single dose, not only an increase in the agglutinins, but also a marked increase in the bacteriolytic power of the blood. In this paper the authors suggested the use of vaccine to limit the spread of epidemics in civil life and in armies during war.

A short time before Pfeiffer and Kolle’s results were announced Sir A. E. Wright, at that time professor in the Royal Army Medical College at Netley, England, published a paper entitled “On the Association of Serious Hemorrhages with Conditions of Defective Blood Coagulability” (28), and in the course of his experimental work on this subject he inoculated 2 men with killed typhoid bacilli. The inoculation seems, however, to have been an incident in a research upon another subject. It served, nevertheless, to demonstrate the harmlessness of inoculating man with dead typhoid bacilli. The following year, 1897, he reported upon the inoculation of 17 persons, and the resultant changes in the blood serum produced by the immunization. It is in this paper that Wright mentions Haffkine’s suggestion to him, made a year previously, that the method of vaccination with bacterial cultures, which had been so successfully used in the prophylaxis of cholera in India, might be applied to the prevention of typhoid fever. This publication makes it clearly evident that Wright had become convinced of the value and practicability of prophylactic inoculation, since he, at that time, suggested its use among physicians, surgeons, and the attendants of hospitals, and also recommended it for armies.

The present campaign of vaccination against the disease dates from the publication of this paper. To be sure, it had previously been suggested by other investigators, but with little result. Wright continued his work with enthusiasm both in India and Great Britain. About 4,000 men of the British Indian Army were inoculated by him in 1898 with excellent results (29). Col. Leishman (10) had reported upon the inoculation of about 100 of the attendants at the Barming Asylum, Maidstone, which were made about this time; and here, too, the results were highly encouraging, since no cases occurred among the inoculated. This was in marked contrast to the large number appearing among the unprotected. Soon after, in 1900, came the Boer War, when Wright convinced the War Office of the desirability of using prophylactic immunization upon the English troops. Voluntary inoculations were authorized, and Wright,
assisted by Leishman, prepared some 400,000 doses of vaccine; though it is believed that not more than 100,000 men received one or more doses. Where it was possible the troops were inoculated before leaving England; yet many received the prophylactic while en route to South Africa, or in the field after arrival.

Regarding the 100,000 men reported to have received one or more inoculations no complete statistics have yet been published, and it is improbable that they will ever appear, as the extreme difficulty of collecting statistical data under such conditions can be readily appreciated. We do know, however, that there were 57,684 cases of typhoid fever with 8,022 deaths among an army of 380,605 (15). This gives a morbidity rate of 151.56 per 1,000, and a mortality rate of 21.08, ratios which differ but slightly from our own in the Spanish War, where no vaccine was used.

This is shown in the following table:

<table>
<thead>
<tr>
<th></th>
<th>Total strength</th>
<th>Cases</th>
<th>Ratio per 1,000</th>
<th>Deaths</th>
<th>Ratio per 1,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>English Army, Boer War, 1900 to 1903</td>
<td>380,605</td>
<td>57,684</td>
<td>151.56</td>
<td>8,022</td>
<td>21.08</td>
</tr>
<tr>
<td>American Army, Spanish War</td>
<td>107,973</td>
<td>20,738</td>
<td>192.6</td>
<td>1,580</td>
<td>14.62</td>
</tr>
</tbody>
</table>

Wright attempted to collect statistics of typhoid fever both among the inoculated and the unprotected; but as his figures cover much less than half of the number of troops employed they failed to carry conviction.

His results are set forth in the following table:

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Cases</th>
<th>Ratio per 1,000</th>
<th>Deaths</th>
<th>Ratio per 1,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinated</td>
<td>19,069</td>
<td>226</td>
<td>11.84</td>
<td>39</td>
<td>2.04</td>
</tr>
<tr>
<td>Unvaccinated</td>
<td>150,231</td>
<td>3,739</td>
<td>24.88</td>
<td>?</td>
<td>?</td>
</tr>
</tbody>
</table>

He considered the incidence of the disease was diminished about one half, and the mortality even more; but his conclusions, based, as they were, upon incomplete returns, were not accepted by his colleagues in the service, and the whole matter was in considerable confusion. It was made still worse by the publication of unfavorable reports, some asserting that
the vaccine did no good, others maintaining that it actually increased the number of cases and deaths (4).

As a result the British War Office suspended the practice of inoculation and appointed a commission to reinvestigate the whole question. This may be said to terminate the first period in the history of the subject, and at its conclusion quite naturally we find the entire procedure viewed with scepticism.

In South Africa antityphoid vaccination had undoubtedly failed to give the hoped-for protection. To explain the relative failure Wright brought forward the doctrine of the negative phase. From experience gained in making determinations of the opsonic index during the course of various infections and after the administration of vaccines he concluded that there was a period during which the content of opsonins in the blood was decreased, and that this drop in the curve occurred after the administration of each dose of vaccine; this was called the negative phase in the progress of immunization. If the dose were not repeated too early, or too large a dose administered, the negative was followed by a positive phase leading to the high tide of immunity. A corollary of this state was a temporary increase of susceptibility to infection so long as the opsonic content of the blood remained below the normal. Wright believed this condition occurred in typhoid, and advised against vaccination when the individual might be exposed to infection before the immunization had been accomplished. Most of the inoculations in South Africa having been made under such conditions, this theory gave a plausible explanation of the poor results obtained. For a time the idea of a dangerous negative phase gained credence throughout the medical world (18).

Subsequent experience has proved the fallacy of this idea of increased susceptibility, and has also furnished the true explanation of the poor results. For these advances we are indebted to the work of Col. Sir Wm. B. Leishman and his assistants (10). In a series of publications in the *Journal of the Royal Army Medical Corps* are related the various modifications made by Leishman of the original Wright vaccine, the most important of which was the change in the temperature used to kill the cultures. For many years 60° C. for one hour had been considered the thermal death point for the bacillus typhosus, but from studies made in Leishman's laboratory it was found that 53° to 54° C. was sufficient, since which time the higher temperature has not been used. In the Harben Lecture Leishman informs us of the method used in preparing the vaccine for South Africa, and it appears that not infrequently even higher temperatures than 60° C. were used, and that, in his opinion, much of the vaccine sent to South Africa had been rendered practically inert from overheating during its preparation. The poor results of the Boer War were due, therefore, not to a negative phase of increased susceptibility, but simply to failure of a defective vaccine to confer sufficient immunity.
German experience with antityphoid vaccine has been described in considerable detail, and (24) the results may be summarized briefly. Typhoid fever had prevailed extensively among the German colonial troops in southwest Africa during the Herero Rebellion.

The usual sanitary measures had all been applied, but without material results. The military authorities referred the matter to Prof. R. Koch for solution, and it was in accordance with his recommendations that vaccination of all possible volunteers was undertaken. A vaccine was prepared at the Institute for Infectious Diseases in Berlin, and about 7,000, or rather less than one half, of the troops volunteered for treatment. Judged by present standards the dose used was large, and the resultant reactions severe. The amount of protection conferred was only enough to reduce the number of cases among the vaccinated to about one half, and the death rate rather more, as the disease, when it occurred, was not so fatal among the vaccinated.

American experience dates from 1908, when the writer was delegated by Gen. Robert M. O'Reilly, at that time chief of the medical service of the Army, to investigate the subject in all its aspects. A visit was made to Col. Leishman's laboratory at the Royal Army Medical College, London, and to the Institute for Infectious Diseases, Berlin, for the purpose of studying the methods already in use. On returning to this country a method was elaborated for our own service, which combined parts of both the English and German methods.

**Preparation of Vaccine.**—The American vaccine, as finally decided upon, is prepared as follows: It is made from a single strain of bacillus (Rawlings), and the culture is grown on agar in flasks for eighteen hours. At first, when small quantities only were needed, test tubes were used, but as the quantities increased Kolle flasks were substituted, each with an agar surface equivalent to twelve tubes.

The culture used is plated out—a dozen colonies are fished on to double sugar-tubes, and from these macroscopic agglutinations are made. Any culture which fails to develop the characteristic appearance on double sugar, or to give a good agglutination, is discarded; from the remaining cultures agar slants are inoculated and the next day emulsified in a small quantity of broth; with this thick emulsion the Kolle flasks are inoculated by means of a large swab. If they show no contamination after eighteen hours' incubation the growth is washed off in a small quantity of salt solution, and, while a sample is being counted, the thick suspension is heated in large flasks in a water bath for one hour at 53° to 54° C.

The killed vaccine is diluted with large quantities of salt solution until the desired concentration, 1,000,000,000 to the cubic centimeter, is obtained. Finally 0.25 per cent. of tricresol is added as a matter of safety. After aërobiç, anaërobiç, and animal tests have been made the
vaccine is put up for shipment in hermetically sealed ampoules of normal glass.

The aerobic and anaerobic tests for sterility are made with large quantities of vaccine, several cubic centimeters to each tube and plate; the animal tests consist in the inoculation of a mouse and guinea-pig with 0.5 and 1.5 c. c. for the exclusion of tetanus spores, and a rabbit with three doses at ten-day intervals to determine the immunizing power of the vaccine. The average titre of the agglutinating rabbit serum obtained with the last eighteen batches of vaccine after thirty days was 1 to 18,000.

Morphological tests of purity, using Gram's stain, are made at each stage of preparation, and a few lots of vaccine have been discarded because of contamination with the bacillus subtilis group, but none have ever been rejected because of the animal tests. They are continued, however, because of the occurrence of a number of deaths from tetanus in India after the administration of plague vaccine. In one case a batch was discarded because it failed to produce good agglutinations in the rabbit test.

We have used agar cultures because of the ease of detecting contamination and to avoid the injection of extraneous materials contained in fluid media.

The vaccine is killed by heat rather than chemicals, using the least amount possible to obtain sterility, and it is protected against subsequent contamination by tricresol.

Our vaccine is essentially the whole body of the bacillus typhosus, changed as little as possible in killing, suspended in a convenient quantity of salt solution. Such a vaccine has the merit of simplicity, is readily and easily prepared, and is constant in quality.

The vaccine has never been kept in stock for any length of time, but is prepared as needed, and none over six months old has been used. It should be stored in the dark at refrigerator temperature until used. Antibodies in good quantities have been produced in animals with vaccines 1½ and 2 years old, but it would probably be poor economy to depend upon old vaccines.

**Directions for Use of Vaccine.**—Three doses are given at 7- to 10-day intervals; the first dose contains 500,000,000 bacteria, the second and third 1,000,000,000 contained in 0.5 c. c. and 1.00 c. c. of fluid. In army practice the 10-day interval is used as most desirable, but in civil practice the 7-day interval is often more convenient; thus bringing the three doses on three successive Saturday afternoons.

Experience has shown that the most suitable hour of the day for vaccinating applicants is late in the afternoon, since the local and general reactions do not usually appear until 4 or 5 hours after, at which time the patient is ready to retire, and by morning the entire reaction may have passed. It is wise to caution against active exercise, such as riding or
tennis, and also against the use of alcohol in any form, since both tend to aggravate the condition.

The vaccine is injected subcutaneously, and not into the muscles nor into the skin; this is necessary to secure slow absorption; deep muscular injections, because of the rapid absorption, are more apt to produce severe reactions and pain on movement.

The best location for the injection is the outer surface of the arm over the insertion of the deltoid muscle, where the subcutaneous tissue is abundant. Sterilization of the skin is secured by tincture of iodin.

In the army none but the healthy are immunized, any illness automatically postponing the vaccination. Postponement, however, rarely occurs, as only healthy men are accepted for service. In civil life conditions are different, and it may be necessary at times to immunize invalids. Each case must be considered on its own merits, and by using a greater number of smaller doses it is probable that many not in good health may be safely immunized. The routine test, of course, of a successful immunization is the presence of a good Widal reaction.

Reaction.—Each dose of vaccine is followed by a local reaction which varies little either with the size of the dose or the idiosyncrasy of the individual.

Usually there is a red and tender spot about two inches in diameter at the point of inoculation. This first appears in 6 to 8 hours and reaches its full development in about 12; it then gradually subsides, and disappears, as a rule, in 48 to 72 hours. It happens occasionally, especially in children, that there is little or no local reaction, but this is a rather rare occurrence. Occasionally the red and swollen area may be quite extensive and extend from above the point of inoculation to the elbow or even halfway to the wrist. At times it also extends upward to the axilla, and the lymph nodes may be swollen and tender on pressure. The symptoms referable to glandular swelling disappear in about 24 hours and are never followed by permanent enlargement or suppuration.

Such extensive local reactions are not particularly painful, and the men are able to use the arm for light work without discomfort; it has never been necessary to use any local application or to place the arm in a sling, and recovery occurs about as quickly as after the usual reaction. This type of reaction is fortunately quite rare.

At the site of inoculation a small, hard, bullet-like nodule may occasionally persist for several weeks before subsiding; no treatment is necessary, as it invariably disappears, leaving no sign.

The general reaction varies in its symptoms much more than the local. In children and in many adults it may be truly said to be absent. The milder form is characterized by a transitory headache and a feeling of weariness lasting from 2 to 3 hours to a day. Slightly more marked general reactions are evidenced by considerable headache and a decided
feeling of lassitude lasting until about noon of the following day. Occasionally there are chilly sensations without much, if any, rise of temperature. A few men have complained of nausea or diarrhea lasting for a few hours to a day. In the average case the mild reaction resembles the feeling of discomfort which precedes an acute cold in the head.

Moderate reactions are those characterized by a rise of temperature varying from 101° to 103° F. Chills may occur, and the symptoms described above may exist in more pronounced form. Moderate reactions follow about 2$\frac{1}{2}$ per cent. of all doses, occurring with about equal frequency after the first and second doses, but much less often after the third dose.

A reaction producing a temperature of 103° F. or over is classed as severe. In many instances there is also a chill or chilly sensations, with more or less headache, nausea, vomiting, or herpes labialis; in the early days when large doses were administered albuminuria was occasionally present after severe reactions; to-day it is extremely infrequent.

It has already been stated that active exercise or alcoholic indulgence may determine a severe reaction; deep injections into the muscle, or wholly or partly into some vein, permitting of quite rapid absorption, are believed to be responsible for the severe reactions which come on almost immediately after inoculation. They are easily prevented by remembering the injunction to introduce the vaccine in every case subcutaneously; when the hypodermic injection is properly given the dose causes a visible and palpable subcutaneous swelling for a few minutes. For the other severe reactions there is no better explanation than the supposition of great susceptibility of the individual to the bacillus typhosus; and it is reasonable to believe that such individuals would, if infected, suffer severely from typhoid fever.

The general reactions following the first 128,903 doses administered to soldiers have been tabulated, and show that the severe type of reaction occurs after only one to three doses per thousand.

<table>
<thead>
<tr>
<th></th>
<th>Number of doses</th>
<th>Reaction, absent</th>
<th>Reaction, mild</th>
<th>Reaction, moderate</th>
<th>Reaction, severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>First dose</td>
<td>45,680</td>
<td>68.2%</td>
<td>28.9%</td>
<td>2.4%</td>
<td>0.3%</td>
</tr>
<tr>
<td>Second dose</td>
<td>44,321</td>
<td>71.3%</td>
<td>25.7%</td>
<td>2.6%</td>
<td>0.2%</td>
</tr>
<tr>
<td>Third dose</td>
<td>38,902</td>
<td>78.0%</td>
<td>20.3%</td>
<td>1.5%</td>
<td>0.1%</td>
</tr>
</tbody>
</table>

Examination of the blood serum of men after vaccination has been made repeatedly. Agglutinins begin to appear on the fifth to eighth day and increase rapidly; ten days after the third dose the Widal is often present in dilutions of 1-5,000, and occasionally the serum shows a titre
of 1-10,000, or even 1-20,000. Only rarely does it fail to exceed 1-600. The rise in opsonins follows quickly, and their increase is quite as striking as the development of agglutinins. Wright's method of estimating the opsonic index is inapplicable in typhoid because of the lytic and agglutinating action of the undiluted serum upon the bacillus typhosus. Resort was had, therefore, to the dilution method of Neufeld, which proved quite simple and satisfactory. The serum is diluted as for agglutination tests, and to equal quantities is added a suitable salt solution suspension of typhoid bacilli; the mixture is incubated at 37° C. for 1 hour. A suspension of guinea-pig leukocytes obtained by injecting aleuronat into the abdominal cavity is then added in equal quantity to each tube, and this mixture is again incubated for an hour; salt solution controls being prepared at the beginning and end of each set of tests. When the incubation is completed smears are made from the sediment in each tube. The phagocytic titre of the serum is determined by ascertaining the highest serum dilution in which the phagocytosis is positive, i. e., in which it exceeds the spontaneous phagocytosis occurring in the controls. Perfectly uniform and consistent results have been obtained by this method. The phagocytic titre is never so high as the agglutinative, nor does it remain up as long; but it has always been well marked and quite constant. A titre of 1-1,000 or 1-2,000 is quite common, while the curve occasionally rises to 1-5,000 or even 1-6,000. It drops at first rapidly and then more slowly, but may still exceed the normal after the lapse of a year.

Other vaccines have from time to time been proposed. The older vaccines of Neisser, Shiga, Bassenge-Rimpau, and Wassermann are merely of historic interest (24). The English and American vaccines are refinements of those of Wright and of Pfeiffer and Kolle. In France the vaccine of Vincent has been used most frequently (25). It is prepared as follows: Several strains isolated in the neighborhood in which the vaccine is to be used are grown on agar twenty-four to forty-eight hours; the growth is taken up in salt solution and kept at 37° C. from two to four days; after centrifugation the supernatant fluid is sterilized by being shaken with ether, which is then allowed to evaporate. Three or four injections are given at short intervals. The results obtained are excellent, and will be referred to later.

Metchnikoff and Besredka in 1911 proposed the use of a living sensitized vaccine (16). They have conducted the most extensive investigation of recent years, using chimpanzees as test animals. They found that in these animals killed vaccines were powerless to prevent typhoid fever when overwhelming doses of infectious material were used, but that prophylactic immunization with sensitized living bacilli gave them power to resist even large doses, such as may occur in milk-borne epidemics. Interesting and valuable as this work of Metchnikoff and Besredka undoubtedly is, it nevertheless deals with a limited number of apes, and for
practical purposes cannot, in the opinion of the writer, be compared to
the work in the military service with nearly 200,000 human beings.

It is evident from Tables VI and VII and Figures 1, 2, and 3, that
our present vaccine is conferring immunity in as great a degree as has
ever been done by any vaccine. It is certain that in the military service
typhoid prophylaxis is quite as successful as vaccination against small-
pox, our old ideal of what a prophylactic measure should accomplish. It
is evident from this that the opinion held by many scientists that living
vaccines and viruses are superior to dead vaccines, and that a high degree
of immunity can only be conferred by the use of living vaccines, must be
reconsidered at least so far as typhoid fever and small-pox are concerned;
our experience has definitely demonstrated that the immunity conferred
by dead typhoid bacilli is in no way inferior to the immunity against
smallpox conferred by living vaccine virus. Sensitized vaccine in this
country has been prepared and used by F. P. Gay, and its use in institu-
tions reported upon by Force (7), who used a sensitized, killed vac-
cine prepared for him by Gay in the following manner:

"The 'Army' strain (long cultivated on laboratory media) of B. ty-
phosus was used. A 24-hour bouillon culture was planted on ten large,
flat-sided (Blake) bottles of agar, incubated at 37.5° C. for 36 hours.
The growth was then washed off in sterile salt solution, 100 c. c. being the
total volume. One c. c. of a strongly agglutinating serum (1-20,000) was
added to this emulsion, and it was allowed to stand one night in the ice-
box. Equal parts of absolute alcohol were then added to the suspension,
the organisms quickly flocculated out completely, and after centrifugaliza-
tion in a high-speed centrifuge the supernatant fluid was poured off and
the residue transferred to a sterile evaporating dish and dried in a partial
vacuum over night. The residue was then scraped off carefully to avoid
any contamination, put into a grinder and ground for one hour. This
fine bacterial powder when kept sterile can be used at any time, making up
a suspension of 1/16 mg. to 1 c. c. carbolated .85 per cent. salt solution."

Force believes the immediate general and local reactions are milder
than those following unsensitized vaccines, a statement we have been
unable to confirm. Little is yet known about the degree and duration of
the immunity conferred by living or killed sensitized vaccines. These
are comparatively new, and it may be well to summarize the reasons
advanced for using them. The first, that good protection cannot be ob-
tained from unsensitized vaccines, falls to the ground, now that American
army experience has demonstrated the contrary. The second reason, that
the reaction is less severe, is still undecided. In a small series of inocu-
lations carried out upon physicians at the Army Medical School the sen-
sitized vaccine produced at least as severe reaction as the unsensitized.
Further investigation is required to demonstrate the degree and duration
of the protection conferred.
Results Obtained in the American Army.—Vaccination was voluntary during 1909, 1910, and the greater part of 1911, since which time it has been compulsory for all members of the service under 45 years of age. In 1909 volunteers were quite difficult to obtain, the greater number being members of the Medical and Hospital Corps of the Army, together with their families, friends, and servants. At the end of that year 1,887 persons had been immunized, most of whom received three doses of the prophylactic. The following year, 1910, volunteers were easier to obtain, and 16,000 persons were treated. During the first part of 1911 volunteers continued to present themselves in increasing numbers, until finally immunized men came to be present in practically every garrison in the United States proper. The measure was no longer strange to the Medical Corps, nor to the enlisted personnel of the Army. We noticed, however, as with all voluntary measures, a great inequality in different garrisons, depending upon the interest and enthusiasm, or lack of it, of the surgeon and the commanding officer.

During the preliminary period of voluntary vaccination records of some 20,000 cases had been collected, clearly demonstrating the safety of the method. It caused comparatively few severe reactions, and no vaccination, no matter how severe the immediate reaction may have been, was followed by any permanent injury to the individual. The degree of immunity conferred, as judged by the usual laboratory tests, was identical with and equal to that following an attack of typhoid fever. The comparative absence of typhoid fever among vaccinated troops, as compared with the unvaccinated, was beginning to confirm the tests for immunity made in the laboratory. (See Table VI, years 1909, '10, and '11.) There was, therefore, abundant proof that the vaccine used in the Army was both harmless and effective.

The introduction of compulsory vaccination occurred in March, 1911, upon the mobilization of a maneuver division in Texas. For the reasons already given it was apparent that it was feasible and practicable to vaccinate the entire 20,000 men in the field. That it was also desirable was immediately apparent from an examination of the reports of typhoid fever in the Spanish-American and other recent campaigns.

<table>
<thead>
<tr>
<th></th>
<th>Strength</th>
<th>Typhoid cases</th>
<th>Typhoid deaths</th>
<th>Killed in action or died of wounds</th>
<th>Died of disease</th>
<th>Wounded</th>
<th>Missing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Franco-German War, German Army</td>
<td>73,393</td>
<td>6,965</td>
<td>28,269</td>
<td>15,240</td>
<td>68,498</td>
<td></td>
<td>12,854</td>
</tr>
<tr>
<td>Spanish-American War, American Army</td>
<td>107,973</td>
<td>20,738</td>
<td>1,580</td>
<td>243</td>
<td>2,665</td>
<td></td>
<td>1,446</td>
</tr>
<tr>
<td>Boer War, British Army</td>
<td>380,005</td>
<td>57,684</td>
<td>9,022</td>
<td>7,702</td>
<td>13,250</td>
<td>22,929</td>
<td></td>
</tr>
<tr>
<td>Russo-Japanese War, Russian Army</td>
<td>17,033</td>
<td>34,000</td>
<td>1,300</td>
<td>141,800</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table IV gives all available information regarding losses from typhoid fever in comparison with losses from other causes in four modern wars, and demonstrates how imperative it was that every possible effort should be made to prevent any recurrence of such epidemics.

The manner in which typhoid fever became epidemic in military camps in 1898 is well known, and the military authorities realized fully that in spite of much improved measures of camp sanitation the disease might again prevail sufficiently to handicap some portion of our forces should they be called upon for actual warfare. It was with full confidence in the measure, and a firm conviction as to its efficacy, that vaccination was made compulsory for the maneuver division.

The immunization of the 20,000 men in the field on the Southern border was carried out promptly and without any special difficulties. The single complication reported was the development of a musculospiral neuritis in one man.

The results obtained are shown in the following table, in which the camp at Jacksonville, Fla., in 1898, is compared with the camp at San Antonio, Texas, in 1911:

<table>
<thead>
<tr>
<th>1898, Spanish-American War, Camp at Jacksonville, Florida</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of troops</td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td>10,759</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>1911, Camp at San Antonio, Texas</th>
</tr>
</thead>
<tbody>
<tr>
<td>12,801</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>........................</td>
</tr>
<tr>
<td>........................</td>
</tr>
<tr>
<td>11</td>
</tr>
</tbody>
</table>

At Jacksonville there were assembled 10,759 men, among whom there were 1,729 undoubted cases of typhoid, and including those in which a diagnosis of typhoid was probable there were 2,673 cases, with 248 deaths. This camp lasted approximately as long as the camp at San Antonio in 1911; both camps were situated in about the same latitude, and each had artesian well water of excellent quality, yet in 1898 there were over 2,500 cases of typhoid fever, with 248 deaths, and in 1911 only 2 cases, with no fatalities (12). We know that the immunity was not due to lack of exposure, since there were reported to the health office 49 cases of typhoid fever, with 19 deaths, among the civil population of the city of San Antonio during the period of encampment.

Soon after the completion of the successful vaccination of this division it was decided to immunize all army recruits at the time of enlistment into the service. This was ordered in June, 1911, since which date
all men on joining the service are vaccinated against small-pox on one arm and against typhoid fever on the other. Only on rare occasions has it been necessary to postpone the second or third doses of the typhoid prophylactic because of vaccinia. Some two to three thousand recruits have been immunized monthly since June, 1911.

The last step was the extension of compulsory prophylaxis to all persons in the service under 45 years of age, and this was ordered on September 30, 1911. In the United States proper the order was not fully executed before January 1, 1912, and in the Philippines not until the first quarter of 1912.

The full effect of these measures can most clearly be set forth in tables and charts of the typhoid fever experience of the army year by year. It is necessary to a correct interpretation of these tables to remember that voluntary immunizations began on a small scale in 1909; that compulsory vaccination was introduced gradually in 1911, but did not include the entire army until 1912.

There are three standards by which to judge of the degree of improvement: the number of cases admitted to sick report, expressed as the admission rate per 1,000 of mean strength; the number of deaths, expressed in the same manner; and the constantly non-effective rate, which is a statement of the average number of men in each 1,000 incapacitated for duty by typhoid fever each day during the year. It is generally acknowledged that the constantly non-effective rate is the truest measure of the gain or loss of efficiency from any or all causes.

Table VI gives all data pertaining to enlisted men stationed within the continental limits of the United States. There is a most decided and significant drop in the ratios for cases and deaths in 1911, 1912, and 1913.

<table>
<thead>
<tr>
<th>Year</th>
<th>Mean strength</th>
<th>Absolute cases</th>
<th>Number of deaths</th>
<th>To each 1,000 soldiers of the command, the ratios are:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>For cases</td>
</tr>
<tr>
<td>1904</td>
<td>43,940</td>
<td>247</td>
<td>12</td>
<td>5.62</td>
</tr>
<tr>
<td>1905</td>
<td>42,834</td>
<td>153</td>
<td>13</td>
<td>3.57</td>
</tr>
<tr>
<td>1906</td>
<td>40,621</td>
<td>230</td>
<td>12</td>
<td>5.66</td>
</tr>
<tr>
<td>1907</td>
<td>35,132</td>
<td>124</td>
<td>7</td>
<td>3.53</td>
</tr>
<tr>
<td>1908</td>
<td>46,316</td>
<td>136</td>
<td>11</td>
<td>2.94</td>
</tr>
<tr>
<td>1909</td>
<td>57,124</td>
<td>173</td>
<td>16</td>
<td>3.03</td>
</tr>
<tr>
<td>1910</td>
<td>55,680</td>
<td>129</td>
<td>9</td>
<td>2.32</td>
</tr>
<tr>
<td>1911</td>
<td>55,240</td>
<td>44</td>
<td>6</td>
<td>0.80</td>
</tr>
<tr>
<td>1912</td>
<td>58,119</td>
<td>15</td>
<td>2</td>
<td>0.26</td>
</tr>
<tr>
<td>1913</td>
<td>50,608</td>
<td>2</td>
<td>0</td>
<td>0.03</td>
</tr>
</tbody>
</table>
RESULTS IN THE AMERICAN ARMY

The significance of the statistics set forth in this table are more easily appreciated when exhibited graphically, and Figures 1 and 2 have been prepared for this reason.

Table VII exhibits the number of cases and deaths occurring each year in the United States (continental) among both officers and men. It shows, also, the number, so far as ascertainable, infected before enlistment, and the number of cases and deaths occurring among the vaccinated each year since the introduction of vaccination.

**TABLE VII**

*Showing the Number and Proportion of Typhoid Fever Cases Contracted before Enlistment and among the Protected (United States Proper only) Officers and Enlisted Men*

<table>
<thead>
<tr>
<th>Year</th>
<th>Total cases</th>
<th>Total deaths</th>
<th>Infected prior to enlistment</th>
<th>Among the vaccinated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Number of cases</td>
</tr>
<tr>
<td>1909</td>
<td>173</td>
<td>16</td>
<td>?</td>
<td>1</td>
</tr>
<tr>
<td>1910</td>
<td>129</td>
<td>9</td>
<td>?</td>
<td>4</td>
</tr>
<tr>
<td>1911</td>
<td>44</td>
<td>6</td>
<td>?</td>
<td>7</td>
</tr>
<tr>
<td>1912</td>
<td>18</td>
<td>3</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>1913</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

Table VIII differs from VI in including all persons in the service, officers as well as men, whether stationed at home or abroad. It covers the last ten years for which figures are available; this table includes a statement of all cases and deaths from typhoid fever occurring among the inoculated. It demonstrates that the improvement was not confined to the United States, but held good throughout the army.

Figure 3 gives graphically the non-effective rate for this disease from 1904 to 1913. The meaning of the term “constantly non-effective rate” and its value for statistical purposes has already been referred to; compared to statistics of morbidity and mortality, it is a quantitative index rather than a qualitative one. It is a statement for each day during the year, of the average number of men per thousand incapacitated for full duty by typhoid fever.

This table should make clear to any large employer of labor or responsible head of any institution or school how antityphoid vaccine would diminish the number of days lost annually from typhoid fever.

From these tables deductions may be made with safety; they are based upon accurate observation by about five hundred physicians of many thousands of men, and are as accurate as only great care can make them. They exhibit a sudden and decided drop in both morbidity and mortality during the past few years, which corresponds with the increase in the use of antityphoid vaccine.
### Table VIII

**Typhoid Fever, 1901 to 1913, for the Whole Army, Officers and Enlisted Men, at Home and Abroad**

<table>
<thead>
<tr>
<th>Year</th>
<th>Mean strength</th>
<th>Cases</th>
<th>Deaths</th>
<th>Occurring among those who were vaccinated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number</td>
<td>Ratio per 1,000 of mean strength</td>
<td>Number</td>
</tr>
<tr>
<td>1901</td>
<td>81,885</td>
<td>552</td>
<td>6.74</td>
<td>74</td>
</tr>
<tr>
<td>1902</td>
<td>80,778</td>
<td>565</td>
<td>6.74</td>
<td>69</td>
</tr>
<tr>
<td>1903</td>
<td>67,643</td>
<td>348</td>
<td>5.14</td>
<td>30</td>
</tr>
<tr>
<td>1904</td>
<td>67,311</td>
<td>293</td>
<td>4.35</td>
<td>23</td>
</tr>
<tr>
<td>1905</td>
<td>65,688</td>
<td>206</td>
<td>3.14</td>
<td>20</td>
</tr>
<tr>
<td>1906</td>
<td>65,159</td>
<td>373</td>
<td>5.72</td>
<td>18</td>
</tr>
<tr>
<td>1907</td>
<td>62,523</td>
<td>237</td>
<td>3.79</td>
<td>19</td>
</tr>
<tr>
<td>1908</td>
<td>74,692</td>
<td>239</td>
<td>3.20</td>
<td>24</td>
</tr>
<tr>
<td>1909</td>
<td>84,077</td>
<td>282</td>
<td>3.35</td>
<td>22</td>
</tr>
<tr>
<td>1910</td>
<td>81,434</td>
<td>198</td>
<td>2.43</td>
<td>14</td>
</tr>
<tr>
<td>1911</td>
<td>82,802</td>
<td>70</td>
<td>.85</td>
<td>8</td>
</tr>
<tr>
<td>1912</td>
<td>88,478</td>
<td>27</td>
<td>.31</td>
<td>4</td>
</tr>
<tr>
<td>1913</td>
<td>90,646</td>
<td>3</td>
<td>.03</td>
<td>0</td>
</tr>
</tbody>
</table>

Further comment upon these tables seems almost superfluous. Each year since 1909 a new low record has been established, and in 1913 the death rate for the first time in any army was reduced to zero.

In the United States Navy similar results have been obtained; the number of cases, deaths and days lost from sickness all show decided improvement. Among approximately 80,000 persons in the navy who have received the full course of vaccine, only seven authentic cases of typhoid fever have developed, and these were characterized by mild symptoms and rapid convalescence. In former years many cases had developed among midshipmen returning to the Naval Academy from holidays spent at home; yet none occurred in 1912, owing to the fact that all cadets had been vaccinated.

Similar results have been obtained in civil life, although no collected statistics are available to show it. Richardson and Spooner (17), Hachtel and Stoner (9), Brannan (2), and many others have used the vaccine both in hospitals and in private practice, so far as known, without untoward results and with good protection.

The State boards of health of Massachusetts, Virginia, South Carolina, and several other states now supply the vaccine gratis in their respective states. New York, Buffalo, Memphis, and many other city health boards have not only provided free vaccine, but have administered it to all volunteers.

The rule of the New York health department is to offer immunization to all members of the household whenever a typhoid patient is found
RESULTS IN THE AMERICAN ARMY

(23). This practice has now prevailed sufficiently long to demonstrate that only good results are obtained. The New York Academy of Medicine has adopted a resolution urging that all persons in any infected family, and any person who has been exposed in any way to the disease, follow all the sanitary precautions usually taken in such cases, and subject themselves to immunization either at the hands of their private physician or of the department of health.

![Chart showing admission rates for typhoid fever, United States (Enlisted Men).]

In addition to American statistics there is abundance of favorable evidence from the British Army in India, where antityphoid vaccination has been in use for a longer period than in any other part of the world.

In France during the past few years, mainly owing to the work of Vincent (25), professor at the French army medical college at Val de Grace, considerable advances have been made, especially among the colonial troops in Tunis and Algeria. Up to November, 1913, Labbé (14) states 100,000 persons had been immunized without any untoward results, and with great reduction in the morbidity and mortality. He intro-
duced, in November, 1913, a bill into the French Senate, which has since become a law, to make vaccination in the army compulsory, as it is in the United States.

Enough evidence has been presented to prove that antityphoid vaccina-
tion is a comparatively simple and, when used on the healthy, a harmless procedure; that it gives rise to a very high degree of immunity closely approaching that conferred by typhoid fever itself and that it has been and easily can be used to immunize large numbers of persons; in fact, its administration to the masses is no more difficult than vaccinia.

**Summary.**—It remains merely to formulate a working plan for future guidance in its use.

Its use is definitely indicated:

1. In the Army, Navy, National Guards of the various states, and all volunteer organizations called into service in time of war.

2. Among the personnel of all hospitals, dispensaries, and Red Cross organizations.

3. In boarding schools, colleges, institutions of all kinds, asylums, prisons, workhouses, and the like.

4. In the camps of pleasure-seekers, explorers, engineers, and contractors. In all these instances its use is sufficiently obvious.

5. Among the inhabitants of cities or districts where the typhoid fever rate is continuously high.

6. Among travelers, especially such as leave sanitary cities for summer vacations in country districts and seaside resorts.

7. Among young adults, young persons, and children. Osler characterizes typhoid fever as a disease of youth and early life, and one which is not infrequent in childhood. It has been shown (20) that children and young persons withstand the immunization rather better than adults; in fact, it rarely interferes with school or play.

The dosage recommended for children is based upon the body weight rather than the age; considering the average adult as weighing in the neighborhood of 150 pounds, a child weighing 50 pounds would be given one-third the dose. Should the fraction of the adult dose be inconvenient to measure in the hypodermic syringe it is better to give a little more rather than less. No harmful results have occurred in several instances in which a considerable overdose was given.

8. Among the members of the household where a case of typhoid fever occurs and all persons who in any way come into contact with the patient.

9. Voluntary vaccination of the non-immune population on the occurrence of an epidemic of typhoid fever. This has been done by Spooner (22), Hunt (11), Goldman (8), and others.

Hunt has pointed out how much may logically be expected from the use of vaccine during epidemics. In outbreaks due to an infected public
water supply it is now the custom of the health authorities, as soon as the diagnosis is made, to sterilize the water with some form of chlorin. This, of itself, is the best measure to stop further primary cases. It will, however, have no effect upon the chain of secondary cases which follow in the wake of every epidemic. It is these contact cases which can be prevented by vaccination subsequent to the outbreak.

The question of vaccination in the case of those already infected, and in the incubation stage of the disease at the time, arises in this connection. A fair number of instances are known both in and out of the service where typhoid fever developed soon after vaccination, but it is not believed that there is any valid reason for thinking that any harm was done; and in many instances it is possible that the disease was rendered less severe. This is not unreasonable in view of the conclusions of Watters (26) that the use of vaccine in the treatment of typhoid fever is promising, and merits investigation.

The question of revaccination has not yet received a definite answer, since the duration of the immunity conferred by our vaccine is not known (18). The immunity is greatest soon after immunization, and it no doubt gradually diminishes as after vaccination against small-pox (19). In the English service the effective duration of the immunity seems to be only two and one half years (6). Although the prophylactic has now been in use in the American service for over four years, there is as yet no indication of loss of immunity. Further experience will, no doubt, clear up this point. The present practice in the Army is to revaccinate against both small-pox and typhoid fever at the commencement of each enlistment period, which is, at present, once in 4 years. This is done, not because we have definite knowledge that the immunity has disappeared, but for the reason that in the Army it would be unwise to depend upon anything less than the maximum obtainable. The general reactions after revaccination are given in the following table, and are seen to be practically the same as after the original immunization:

**General Reactions Following Revaccination**

*Jan. 30, 1914*

<table>
<thead>
<tr>
<th>Number of doses</th>
<th>Absent</th>
<th>Per cent.</th>
<th>Mild</th>
<th>Per cent.</th>
<th>Moderate</th>
<th>Per cent.</th>
<th>Severe</th>
<th>Per cent.</th>
</tr>
</thead>
<tbody>
<tr>
<td>First dose......</td>
<td>500</td>
<td>359</td>
<td>71.8</td>
<td>127</td>
<td>25.4</td>
<td>13</td>
<td>2.6</td>
<td>1</td>
</tr>
<tr>
<td>Second dose.....</td>
<td>500</td>
<td>382</td>
<td>76.4</td>
<td>95</td>
<td>19.0</td>
<td>23</td>
<td>4.6</td>
<td>0</td>
</tr>
<tr>
<td>Third dose......</td>
<td>500</td>
<td>417</td>
<td>83.4</td>
<td>71</td>
<td>14.2</td>
<td>10</td>
<td>2.0</td>
<td>2</td>
</tr>
<tr>
<td>Total...........</td>
<td>1,500</td>
<td>1,158</td>
<td>77.2</td>
<td>293</td>
<td>19.5</td>
<td>46</td>
<td>3.1</td>
<td>3</td>
</tr>
</tbody>
</table>

The future may indicate that reimmunization against typhoid need not be done more often than revaccination against small-pox, i. e., in
SUMMARY

childhood, youth, for military service and upon exposure to infection.

At one time it was believed that the agglutination reaction would indicate the presence or absence of this immunity. The fallacy became apparent when it was noted that the agglutination reaction usually disappears in from six to eighteen months after typhoid fever itself, although the immunity remains, as a rule, for life.

In conclusion it may not be amiss to recall that vaccination is not the only measure to be used in the suppression of typhoid fever. Good, pure water supplies, proper sewer systems and purification plants, and all other general sanitary measures are imperative, and none should be overlooked. Antityphoid vaccination is a matter of personal hygiene rather than of general sanitation, and is useful in protecting the individual against accidental or unusual exposure or where sanitary safeguards are inadequate. At present vaccination is the only method offering protection against infection at all times and under all conditions. There is no occasion for conflict between the advocates of general and individual prophylaxis; one is as necessary as the other, and no one interested in the suppression of this disease can afford to ignore either.

We have now reached the stage in preventive medicine when it is possible to declare that deaths from typhoid fever are practically unavoidable. Wherever state or municipal authorities fail to provide adequate sanitary safeguards the individual now has it in his power to obtain through vaccination almost absolute protection against infection. There is sufficient proof to justify physicians in any part of the United States in urging upon their clientèle, especially upon the young people and children, the use of the vaccine with just as much confidence and authority as has been used in urging vaccination against small-pox.

[The military experience of the past two years has tended to confirm the conclusions derived from army and hospital statistics during times of peace, concerning the value of antityphoid vaccination as a protective measure against typhoid fever. It has been urged by some that the elimination of typhoid fever from our army following the introduction of antityphoid vaccination was due not so much to vaccination as to improved sanitary conditions. This argument will hardly hold, however, in view of the success in controlling typhoid under the severe conditions of trench warfare on the western front in Europe.

Typhoid and Paratyphoid.—Vaccination against typhoid fever does not seem to protect against infections caused by paratyphoid organisms. During the early months of mobilization in the present war, certain troops were immunized against typhoid only. When these troops entered the field a few months later, typhoid fever was almost entirely absent, but paratyphoid was found in a number of instances. Subsequently paratyphoid vaccine was included with the typhoid for immunization.

Paratyphoid infections are not so common as typhoid, but since the circumstances of infection are similar in these diseases, it is advisable to include paratyphoid A and B with the typhoid organisms in the immunizing vaccine.

Dosage.—The first dose of vaccine may contain 500 millions of killed typhoid bacilli, 250 millions of paratyphoid A and 250 millions of paratyphoid B. The
second and third doses are each double the amount of the first injection, that is, 1,000 millions of typhoid and 500 millions each of paratyphoid A and paratyphoid B. The reactions following the vaccine containing paratyphoid in addition to the typhoid bacilli do not appear to be more severe than those following immunization by typhoid bacilli alone.—Editors.]

REFERENCES

CHAPTER VI

BACTERIAL AND SERUM THERAPY IN TYPHOID AND PARATYPHOID FEVERS

WILLARD J. STONE

TYPHOID FEVER

GENERAL CONSIDERATIONS

The organism recognized as the cause of typhoid fever was described by Eberth in 1880-81. It was first observed by him in the intestinal ulcerations and enlarged spleen incident to the disease, but was not isolated in pure culture until 1884 by Gaffky. The B. typhosus belongs to a group of organisms which bear more or less close resemblance to it, and are widespread in nature. This group comprises the B. coli (Escherich), the B. dysenteriae (Shiga), the B. enteritidis (Gärtner), the B. psittacosis (Nocard), the B. cholerae suis (Salmon and Smith), the B. paratyphosus (Achard and Bensaude), and B. paracoli.

All of these organisms under certain conditions become pathogenic for man. Certain exceptions to this general rule, however, occur. For example, a certain proportion of individuals in close contact with those ill with typhoid fever, such as orderlies and nurses, may eliminate the organisms in the urine and stools for varying periods of time without themselves manifesting past or present symptoms of the disease. Such individuals have been termed “contact carriers.” Their apparent resistance is to be ascribed either to a natural immunity or to an acquired immunity from an earlier unrecognized infection which may have been called “summer diarrhea,” “non-typhoidal enteritis,” or “food or ptomain poisoning.” On the other hand, a natural immunity may exist in some individuals. The condition may be considered to correspond to the partial natural immunity which, for example, most individuals manifest toward the pneumococcus. A large percentage of supposedly normal individuals harbor this latter organism in their bronchial secretions during the winter months, and yet many of them do not develop the disease.

In other words, if certain organisms were always pathogenic for man,
their presence in the body would always mean the production of disease, unless specific immunity to the organism in question had been secured. This obviously does not correspond with the state of affairs since other factors enter into the consideration, such as the question of virulence and the presence of secondary bacteria which may, through disintegration or the elimination of toxic products, influence tissue and cellular susceptibility. Thus the presence of B. coli has been shown experimentally to increase the virulence of B. typhosus. In fact, many clinicians consider with more or less experimental justification that many of the symptoms of typhoid are due to the presence of bacteria associated with B. typhosus, such as B. coli. This organism is ordinarily a harmless saprophyte, which may manifest pathogenic properties.

The susceptibility of certain tissues likewise exerts considerable influence upon the destructive tendencies of certain bacteria. This is shown in the tendency of the members of this group to produce intestinal lesions. In the manifestations of disease produced by the members of this group a bacteriemia is many times present. The destructive lesions are, however, commonly restricted to certain tissues. Thus in typhoid the lungs, liver, and kidneys are usually spared. Parenchymatous changes of moderate grade usually occur in the organs of elimination, such as the liver and kidneys, during any fever caused by infective organisms, but in typhoid do not, as a rule, seriously impair their functions.

The belief is, however, gaining ground that certain phenomena of disease, such as fever and tissue waste, are the results of poisonous protein groups set free during the digestion or splitting of the protein constituents of bacterial cells in the body. This is accomplished in a more or less specific manner by the cellular components of certain tissues. That certain bacteria stimulate certain cells of the body to activity in defense seems established. For example, in typhoid the mononuclear leukocytes, the endothelial cells of serous membranes, and the lymphoid cells of the body appear to be especially active in defense, while the neutrophile polymorphonuclear leukocytes are less active. This is shown in the relative increase in the blood of the mononuclear elements in uncomplicated typhoid fever.¹

The work of Vaughan (135) and his associates has brought into prominence the fact that the harmful influence of most bacteria in the body depends upon the breaking up of the bacterial protein with the liberation of poisonous groups under the influence of certain susceptible tissue cells. When these cells are able to show a protective response the bacterial cells are attacked, with the liberation of the poisonous protein group. The manifestations of the disease are largely due to the liberation of this protein

¹ When complications occur, such as pneumonia, cholecystitis, septic thrombosis of the pelvic, saphenous or femoral veins, septic infarction or rupture of the spleen, septic peritonitis or osteitis, abscess or rupture of the mesenteric glands, abscess of the liver, mastoiditis or intestinal perforation, the leukocytosis which follows is largely due to the increase in the polymorphonuclears.
poison. In typhoid during the incubation stage, when the bacteria are multiplying rapidly in the lymphoid tissue, there are few manifestations of the disease. The symptoms of the disease as recognized by clinicians are ushered in when the tissue cells have developed enough ferment to attack the bacterial invaders and to destroy them. In this process of bacterial proteolysis the bacterial protein is set free and symptoms of poisoning follow, which continue as long as the bacterial protein is being liberated in considerable quantity. The symptoms do not continue, however, until all bacterial cells are destroyed, but are in evidence as long as any considerable quantity of protein poison is being set free. Individuals convalescent from typhoid fever may continue to harbor the B. typhosus in the gall-bladder, urine, and stools for weeks, months, or years, and are known as acute, subacute, or chronic carriers. No considerable quantity of protein poison, however, is being set free, and general symptoms of intoxication, fever, and tissue waste are not in evidence.

The bacterial protein poison set free under the influence of tissue cells in disease can be separated in vitro from certain non-poisonous groups which make up the protein molecule by Vaughan's method, which consists in boiling large quantities of the bacterial cells with a 2 per cent. solution of sodium hydroxid in absolute alcohol. The poisonous toxophore residue when injected into animals produces symptoms of poisoning. It is not a toxin in the true sense, since it does not produce an antitoxin in the animal body. It is merely the poisonous portion of the protein molecule, and is exactly similar in effect to the poisonous portion obtained from other bacteria or other proteins, such as egg white. Jobling and Bull (88) have found that a single intravenous injection of washed typhoid bacilli into dogs produced the symptoms which are characteristic of anaphylaxis. Vaughan has obtained similar results by using a variety of protein poisons. It seems to make little difference from what source the protein poison is obtained. Its effects, when injected in sufficient quantity, are always the same.

It is possible that the predilection of bacteria for certain tissues is due to selective action, which depends upon their ability to multiply to better advantage in some tissues than in others. Thus the B. typhosus selects the blood stream and lymphoid tissues of the body. As an adaptive parasite it may, however, develop in other locations, as in the cerebrospinal fluid, the lungs, the liver, and gall-bladder, the periosteum or brain. That there is selective action in the development of bacteria in the body is well shown in the predilection of the pneumococcus for the lungs, the meningococcus for the serous membranes, the staphylococcus for the skin, the diphtheria bacillus for mucous membranes, and the causative organisms of poliomyelitis and rabies for the tissues of the central nervous system.

1 The word toxin, as here used, refers to poisons elaborated by and during the life of the bacterial cell.
Some bacteria have become adaptive parasites through many generations of growth in the animal body, and are able to find suitable conditions for growth in various tissues. The tubercle bacillus is perhaps the best example of adaptive parasitism.

In any long-continued fever, such as typhoid, the nitrogenous output is always increased, no matter how assiduously the physician endeavors, by an appropriate diet, to make good the waste. In health the anabolism exceeds the catabolism; otherwise cellular equilibrium could not long endure. In any systemic disease produced by bacteria the cellular waste is increased, since it is the unusual activity of the cells and the antibacterial ferments elaborated by them which make possible the destruction of bacteria. On the one hand, the bacteria, through the splitting of their protein molecules, produce poisonous substances inimical to the tissue cells. On the other, the tissue cells in the process of disintegration by bacteria may be split into poisonous and non-poisonous portions such as can be obtained by the digestion of tissues in the test tube.

Tissue waste of itself can therefore be a possible source of danger to the body through its own split products of protein disintegration produced by bacteria. It is not uncommon to see toxic manifestations in typhoid fever subsequent to the typical course of the disease. In many instances the cause is to be found in the tissue waste incident to starvation. In fact, such fever is often called "starvation fever" with evident good reason, since such manifestations often disappear when a more liberal dietary is substituted for the too prevalent milk and lime water régime.

Peptone itself is a poisonous product, and when injected intraperitoneally in animals produces symptoms of poisoning or death. When the protein molecule is acted upon by body ferments, however, in the process of gastro-intestinal digestion, the digestion is carried rapidly beyond the peptone stage to the non-toxic amino acids. When the bacterial cell in the process of protein digestion is carried beyond the poisonous stage under the influence of tissue ferments, and only the non-poisonous residue remains, then the disease process stops. Since it has been shown that it is the non-poisonous or haptophore residue which seems to be instrumental in the production of tissue tolerance or immunity, it follows that the immunity to the disease becomes evident at the time the tissue cells have acquired the ability to elaborate sufficient ferment to carry the digestion of the bacterial cells beyond the poisonous stage.

**BACTERIOLOGICAL AND SEROLOGICAL DIAGNOSIS**

In the present state of knowledge it is well known that the diagnosis of typhoid or paratyphoid fever may depend upon bacteriologic and serologic methods, without which the diagnosis must remain in doubt. This has been especially true during the past five years as the result of an enormous
amount of experimental work. The physicians of two decades ago could make a diagnosis of a continued fever with serenity and without question, while to-day in atypical cases the diagnosis rests upon an adequate serologic and bacteriologic foundation. The differential and bacteriologic characteristics of the important members of the enteritidis group have been given in the chapter dealing with B. coli infections (Chapter VII).

Blood cultures, undoubtedly, are the most reliable aid early in the disease, that is, until the third week. Bacilli can be cultivated in about 90 per cent. of the cases during the first week. The usefulness of the test decreases after the second week, although probably a majority of the cases will show bacilli by the blood culture up to and including the third week. Tebbutt (130) found that, on an average, 70 per cent. of patients with typhoid still show bacilli in the blood stream on the fourteenth day. As has been stated by Todd (132), the blood culture is in a sense complementary to the agglutination test, since the blood culture test decreases as the agglutination test increases in reliability as the disease progresses. Schatz (116), Epstein (43), Bates (14), Kiralyfi (75), and others have found the bacilli in practically all cases during the first week. There seems to be little difference in the results whether one uses blood by finger puncture or by vein puncture during the first week. (Todd, loc. cit., Fornet (44), Schatz, loc. cit.)

Puncture of a vein at the bend of an elbow and the withdrawal of 5 c. c. of blood offers less chance of contamination, and should be preferred as the method of choice. The blood when secured in this manner is then discharged into 10 c. c. of sterile ox bile or into an ox bile medium containing 1 per cent. of glycerin and 1 per cent. Witte's peptone. At the end of 18 to 24 hours' incubation, plates are made upon a modified Hesse's medium,¹ and the cultures identified by subculture as to (1) motility, (2) absence of indol, (3) absence of acid and milk coagulation, (4) absence of gas production in gelatin stab, (5) non-liquefaction of gelatin, (6) agglutination with a known antiserum, whose titre has been determined (see under Agglutinins), and (7) sugar-fermentation tests. The organisms most difficult to identify in ordinary clinical work are B. paratyphosus, B. coli, and B. paracoli. As a rule, no great difficulty is encountered. Occasionally, however, variations in type are found to occur, which make the differentiation a matter of much labor.

In this connection the work of Soberneheim (123) and of Soberneheim and Seligman (124) may be mentioned. They have been able to show that the biologic and cultural characteristics of B. typhosus can be changed to

¹ Hesse's medium, azolitmin lactose agar, as modified by Stokes and Hachtel, is made as follows:

Agar 6.0 dissolved in aq. dest. 500.0. Then add peptone 10.0, Liebig's ex. of beef 5.0 and sodium chlorid 8.5 dissolved in 500 c. c. water at 55° C. To the mixture add lactose 10.0 and glycerin 50.0. Neutralize the solution and add 1 per cent. azolitmin which has been dissolved in water by boiling.
correspond to those of the type of B. enteritidis and to paratyphoid A and B or paracolon by variations in the media and environment. The mutation of other organisms as to type may occur by variations in media and environment. Lamar (79) and Rosenow (109), each working with the pneumococcus, were able to change its Gram-positive characteristics by varying the surface tension of the bacterial cell, either by autolysis or by suspension in sodium oleate solution. Sobernheim (loc. cit.) has shown that a typhoid serum will sometimes react with certain strains of B. enteritidis. These facts may, of course, be confusing to accurate bacteriologic diagnosis, but need not interfere with a "group diagnosis" covering infection with some of the members of the enteritidis group.

Epstein (loc. cit.) found that in blood cultures in typhoid fever good results were obtained by the use of 2 per cent. glucose bouillon and 2 per cent. glucose agar media. He found that the various bile media, contrary to the general experience, were not as reliable as the media mentioned above. Bates (loc. cit.), in an interesting review of blood culture and agglutination tests in 68 cases of mild atypical typhoid fever, found that about 60 per cent. gave positive blood cultures, while only 46 per cent. gave positive agglutination tests. He considers atypical typhoid of short duration much more common than is generally supposed. Kiralyfi (loc. cit.) has succeeded, in a bacteriologic study of the bile in cholecystitis, in obtaining pure cultures of B. typhosus from the bile in typhoid fever. The bile was obtained by an oil breakfast of 250 to 300 c. c. of sterile olive oil introduced through a sterile tube after lavage of the stomach with sterile water. The oil was removed in one-half hour through a sterile tube. Sputum and expectoration must not be swallowed during the period when the oil is in the stomach. The oil and bile-tinged fluid which have entered the stomach are easily separated, the oil rising to the top of the residue. Bacteriologic tests of the bile showed B. typhosus in three cases of typhoid. This more or less complicated procedure may be of value in furthering the diagnosis in obscure cases, especially in the diagnosis of chronic typhoid infection of the gall-bladder; but as a rule the clinical symptoms pointing to lodgment and perpetuation of the bacilli in the gall-bladder, subsequent to typhoid, are usually characteristic, and such elaborate procedures as outlined above are unnecessary.

MacNeal and Chace (86), in a study of the bacteriologic contents of the duodenum obtained through the use of the Einhorn duodenal tube, found that normally the fasting duodenum is almost free from living micro-organisms. In various gastro-intestinal disturbances the number of living organisms is greatly increased. In one patient, with relapse in typhoid fever, they were able to recover B. typhosus from the duodenal fluid. Einhorn (41) has also mentioned the recovery, by means of the duodenal tube, of the B. typhosus from the duodenum of a typhoid carrier.

At about the time of defervescence the organisms disappear from the
blood stream, but reappear in true relapse. Coleman and Buxton (29) have stated that post-typhoidal elevations of temperature may be due to the discharge of limited numbers of bacilli into the blood stream from the spleen and other organs. These authors apparently still cling to the older idea of the liberation of endotoxins (Pfeiffer) in the blood stream as the cause of the fever. If, however, the cause of the fever depends, as before mentioned, upon the liberation of sufficient poisonous protein due to the disintegration of bacterial cells (or tissue cells as a result of body waste), the bacteria need not necessarily be in the blood stream. In fact, such destruction could occur in any of the tissues. There is no proof that bacteria when destroyed in the blood stream are more poisonous than when destroyed in other tissues. The matter of relapse is probably a quantitative rather than a qualitative reaction; that is to say, when the bacterial protein poison is liberated in sufficient quantity due to the rapid unrestrained development and destruction of large numbers of bacteria, symptoms, such as fever and enlargement of the spleen, occur as reactive phenomena. When, however, only a decreasingly small number of bacteria are being destroyed each day, as in convalescent carriers without symptoms, insufficient protein poison is elaborated to produce reactive response. In brief, while it is often true that relapse is associated with a new crop of bacteria in the blood stream, this is by no means a necessary part of the process, for bacterial growth may be progressing in the lymphoid tissues. As before emphasized, the fever of a supposed relapse may be due to one of the numerous complications of the disease or to extensive tissue waste due to starvation.

It may be convenient to draw the blood for the culture and agglutination tests in one of the blood capsules of Wright. The finger is cleansed thoroughly with alcohol or painted with tincture of iodin, then punctured with a sterile needle or lancet and the blood collected in the capsule which has been sterilized by holding it for a few moments in an alcohol flame. The ends are then sealed in the flame. The capsule is opened at the time the test is made by filing and breaking above the clot. A drop or two of serum is removed with a sterile capillary pipette for the agglutination test, while the clot is fished out with a sterile platinum wire and planted in 5 c. c. of sterile bile. This clot-culture method gives results very slightly, if at all, inferior to the methods using larger quantities of blood, in the experience of Lyons (85). This is especially true of the first week. Any of the bile media mentioned are of service. Ox bile contains, under normal conditions, little albumin, and hence can be readily sterilized by bringing to 60° C. for an hour on three successive days. Since positive blood cultures can be obtained earlier than positive agglutination tests, this early aid in the diagnosis of typhoid and paratyphoid fever should be utilized. Fornet (loc. cit.) was able to obtain typhoid bacilli from the blood stream in 14 out of 19 cases of typhoid fever long before the agglutination tests
were positive. On the other hand, Busse (24) has been able to cultivate B. typhosus from the blood of one patient with pneumonia and from the blood of three patients with miliary tuberculosis. In none of these were there any symptoms of typhoid during life, and no lesions of typhoid were found at autopsy. He regards such patients as probable typhoid carriers. Such findings are unusual, and may be accidental. The B. typhosus is rarely found in the blood of carriers, and it should be borne in mind that typhoid without intestinal lesions may occur. Many such instances are cited in the recent article by Cummins and Brown (35) on atypical typhoid infection. This article contains an extensive bibliography.

In the bacteriologic examination of the urine of suspected typhoid carriers it is better, if not essential in the case of women, to secure the specimens by catheter. From 0.1 to 1.0 c. c. may then be inoculated over the surface of MacConkey's 1 per cent. bile-salt lactose agar or over the surface of Hesse's azolinmin lactose agar. Plates are then made and the bacteriologic reactions studied as to gas, indol, and acid production, milk coagulation, motility, sugar fermentation, and agglutinative reactions with a known immune serum.

In the examination of stools for B. typhosus, B. coli, normally present, often serve as a source of confusion. Small portions of feces are rubbed up in a sterile mortar with 8 or 10 volumes of sterile salt solution, from which inoculations of varying quantities from 0.1 to 0.5 c. c. are made upon the media mentioned above. Subsequent plating is then carried out and the organisms identified. Negative findings as to B. typhosus have little value, and the examination should be repeated at different intervals in order to be certain of the bacteriologic diagnosis. Since the bacilli are probably intermittently ejected from the gall-bladder into the intestine in typhoid carriers, the stools may only intermittently contain the bacilli.

**Serum Agglutination.**—The adaptation of the Pfeiffer phenomenon of agglutination of bacilli by the corresponding immune serum to typhoid fever was due to the studies of Widal and Grünbaum. Their results were obtained independently, but Widal published first. He showed that comparatively early in the disease the agglutinative power of blood serum should be utilized as important confirmatory evidence of infection with the B. typhosus. This test has become widely used in the diagnosis of typhoid fever, and when positive is specific in the sense that the B. typhosus is the cause of an existing infection, if the individual has not within recent years had an attack of the disease or received within the preceding three or five years a series of antityphoid inoculations. As commonly applied, the following method is used. The blood from the finger or ear is collected in a small test tube or blood capsule, or a few drops are allowed to drop and clot upon a glass slide or cover glass (Wyatt Johnson). The serum is separated from the clot if the former method is used, and diluted 1-25 with normal

1 *Semaine méd.*, 1896, 303.
salt solution in a leukocytometer pipette. One platinum loopful of a
12 to 24-hour bouillon culture of B. typhosus is placed upon a cover glass
and one loopful of the 1-25 serum dilution, which makes a dilution of
1-50. This is inverted over a hanging drop chamber on a slide, the edge
of the chamber having been rimmed with vaselin. Likewise one loopful
of the bouillon culture is added to one loopful of the 1-25 serum dilution
on a cover glass with two loopfuls of salt solution, which results in a
1-100 dilution. These hanging drop preparations are watched for one
hour under the microscope. If the serum is derived from a patient with
typhoid fever, beyond the seventh day of the disease, distinct clumping of
the organisms with loss of motion occurs, according to my experience,
within one-half hour with 1-50 dilution.

Many observers use a 1-30 or 1-40 dilution of serum in making the
test. In at least one-half of the cases agglutination with complete loss of
motion occurs in 1-100 dilution of serum within the half-hour limit. This
reaction becomes evident, as a rule, in the disease about the end of the first
week, but may be delayed until late in the second or third week. It has
been rarely obtained, in my experience, earlier than the fifth day, figuring
the first day of fever from the time the patient takes to bed. Hunt (64)
in a study of typhoid fever found that the agglutinins were present in from
18 to 25 per cent. during the first week but after the fifth day, in 60 to
65 per cent. during the second week, 80 to 90 per cent. during the third
week, and about 94 per cent. during the fourth week. Some typhoid sera
manifest very high agglutinating properties. In fact, it is not unusual to
find human sera after antityphoid inoculation which will agglutinate in
dilution 1-300 to 1-500. Austin and Frothingham (8) have occasionally
obtained a positive reaction in dilutions as high as 1-600 to 1-800. The
agglutinative reaction usually disappears within a few months following
convalescence, but it has been found to persist in some instances for two
years or longer. My own blood serum, following a moderately severe at-
tack of typhoid, would agglutinate the B. typhosus sixteen months later in
dilution 1-60. Some strains of bacilli agglutinate more rapidly than
others, and it is essential that the laboratory worker who makes the test be
familiar with the motility and agglutinative characteristics of the strain
with known typhoid sera. It is essential also that the strain is not sus-
ceptible to the action of normal serum. Variations in agglutinability are
more marked in freshly isolated strains. Some strains of typhoid bacilli
appear to be susceptible to spontaneous clumping in bouillon. Jordan (70)
consequently believes that it is better in testing for agglutinins to use a
suspension of bacilli, from an eighteen-hour-old agar culture, in salt solu-
tion.

As has been stated by Gay and Claypole (49), it is frequently impossi-
ble to identify freshly isolated strains of typhoid bacilli by their agglutina-
tive reactions with potent antiserum until such strains have grown for
a few generations upon artificial culture media. These authors have shown that strains of B. typhosus possess varying degrees of agglutinability, depending upon the media upon which the organisms are grown. For example, an immune serum from a rabbit immunized by blood-agar cultures would agglutinate to its full potency both blood and agar cultures of the organism, while rabbit serum immunized against agar cultures would only agglutinate the organisms grown on agar. The lack of agglutinability of blood-agar strains by rabbit serum immunized to agar strains alone could be changed so that such strains would be agglutinable by transference to the agar medium. These facts are of considerable importance to those doing serologic work in the identification of different strains of B. typhosus. In severe infections in individuals who show little apparent resisting powers the agglutination test may be absent, and as such has been considered by some as an unfavorable sign.

The agglutination test may be performed macroscopically, and a dead suspension of the organisms may be used instead of the living culture. Bass and Watkins (12) have simplified a macroscopic agglutinative reaction which is satisfactory qualitatively in many instances, but lacks quantitatively. This, however, is not a serious objection. Its advantages are simplicity, convenience, and the rapidity with which the test may be performed at the bedside. A suspension of B. typhosus in salt solution, 10,000,000,000 per c. c., is used, to which is added 1 per cent. liquor formaldehyde. A drop or two of suspected blood on one end of a glass slide is diluted with three or four times its volume of water or salt solution. A drop or two of this diluted blood is placed on the end of the slide, and an equal volume of the bacterial suspension is added. The slide is then gently tilted from side to side so as to agitate the mixture. If the reaction is positive a grayish mealy sediment of agglutinated bacilli becomes evident within a minute or two. This appears first in the fluid at the edges. When the reaction is negative no agglutination occurs, and the mixture remains clear. This macroscopic test was positive in about 92 per cent. of their series of typhoid cases.

The persistence of the agglutination reaction after typhoid fever or antityphoid inoculations has been considered by many writers as an index of immunity. It is apparent that other factors are of greater importance in the immunity which follows an attack in most cases. For, while the agglutination capability of the serum diminishes markedly six months after convalescence, the immunity is established in most instances for life. As Moon (94) has stated, "the degree of resistance to typhoid does not run parallel with the agglutination curve." In other words, blood serum may be weakly agglutinative and strongly bactericidal and vice versa. The association of agglutination with an immune serum has become firmly fixed in the minds of most physicians. As stated by Muir and Ritchie (95), "While agglutinative power cannot in itself be taken as the measure of the
degree of immunity, agglutinins and immune bodies are the products of corresponding reactive processes.” This much may be granted, the agglutinative qualities of a given serum appear during the process of immunity or tissue tolerance acquired by the tissue cells under the influence of a given infection, and persist as one of the evidences of the effect of that infection for considerable periods of time.

The agglutinative reaction of typhoid immune serum is specific in the sense that it manifests this property in its highest potency or titre only against the B. typhosus. More or less agglutination may occur with such serum and closely allied bacteria such as B. coli. Although many strains of the latter organism do not react with typhoid immune serum, there are some strains which do. For example, a strain of B. coli which was isolated from the urine of a patient with B. coli bacilluria was not completely agglutinated in one-half hour by the sera of five healthy individuals in dilutions higher than 1 in 5. On the other hand, this organism was readily agglutinated in one-half hour by the sera in dilution 1 in 20 of four individuals who had received within the preceding two years a series of antityphoid inoculations. Other organisms, members of the so-called enteritis group of bacteria, may react the same way. This has been particularly true of the paratyphoid bacillus. Generally, it may be stated that in paratyphoid fever (see below) the blood serum possesses marked agglutinative properties toward the paratyphoid bacillus, much higher dilutions of serum causing more complete agglutination than can be obtained when the B. typhosus is used. Then, too, multiple infections may occur, such as B. coli or B. paratyphosus, with B. typhosus. When intestinal ulceration occurs in the course of typhoid, the avenue of infection is opened to the lymphatics and blood stream. Since the B. coli is a normal inhabitant of the bowel, and since the B. paratyphosus has been found in the bowel when no symptoms of disease were present (Muir and Ritchie), it is easy to understand the manner in which systemic infection by these organisms may occur synchronously with typhoid.

Ocular and Skin Reactions in Typhoid.—In 1907 Chantemesse (27) reported his experiences with an ophthalmic diagnostic reaction in typhoid. The reaction was based upon the theory of cell sensitization to the invading specific bacteria producing the disease, and was similar in effect to the ophthalmic reaction in tuberculosis. He obtained his typhotoxin by precipitating cultures of B. typhosus with absolute alcohol. The precipitate was then powdered and resuspended in aqueous solution so that each drop represented about 0.02 mg. One drop was instilled in the eye. If the patient had typhoid, congestion, lachrymation, and the effusion of a serofibrinous exudate developed within a few hours and persisted for 24 to 48 hours. In normal individuals the instillation produced congestion, lachrymation and exudation of lesser degree, which usually disappeared in 4 to 6 hours, so that at the end of 24 hours no signs of reaction remained.
Chantemesse was able to show that the reaction could be obtained 48 hours subsequent to the subcutaneous injection of typhoid cultures. Kraus (76), who repeated the work of Chantemesse, could not convince himself of the specificity of the reaction. He found that healthy individuals many times responded moderately to the test and that other bacterial extracts produced a similar response in typhoid patients. Austrian (9) has recently applied this test in 75 cases of typhoid, and secured a positive response in 71. He also examined a total of 190 persons normal or ill with disease other than typhoid. In only two instances did a typhoid-like reaction develop. Austrian used a stronger solution than Chantemesse, containing from 1/3 to 1/2 mg. per drop. He believes that the most constant and reliable results will be obtained when the typhoprotein used is secured from a mixture of numerous strains of bacilli. These results seem to indicate that a condition of sensitization or allergy does exist early in typhoid, contrary to the opinion of von Pirquet (101) that such a state does not exist in this disease.

In 1909 Deehan (39) reported a typhoid cutaneous reaction which was positive in a limited number of cases tried by him and negative in a limited number of controls. This work has not to my knowledge been repeated or verified. Kraus had also tried a cutaneous reaction, but was unsuccessful. Two years ago the attempt was made by us to obtain an antigen suitable for a cutaneous reaction in typhoid. The bacilli were grown in 500 c. c. flasks of bouillon for two weeks, filtered through a Berkefeld filter and the germ substance collected. The germ substance was also obtained from bouillon cultures by high speed centrifugation in a large machine. Part of the germ substance was then ground in a mortar and a few drops of benzine added from time to time until an amorphous powder resulted. This was suspended in the proportion of 5 mg. to each c. c. in salt solution containing 0.5 per cent. phenol. Injection of a drop of this suspension intradermally or subcutaneously was followed by a roseolar blush of inflammatory reaction surrounding the point of injection. This occurred in individuals with typhoid as well as in normals and controls. We then precipitated bouillon cultures with absolute alcohol and collected the precipitate, which was dried and resuspended in salt solution containing 0.5 per cent. phenol. This residue produced a less marked inflammatory reaction when injected under the skin, but like the suspension previously mentioned, seemed to be non-specific. It seems reasonable, however, to suppose that a specifically reacting non-toxic substance may be obtained from the typhoid bacillus, which will, when injected, produce a local cellular response in individuals suffering from the disease, and not in normals or controls. The conjunctival cells are apparently sensitized, and it is probable that other tissue cells are also.

Agglutination of B. Typhosus by Tuberculous Serum.—Baetz and Bates (10) have recently stated that in Ancon Hospital, Canal Zone, they
obtained 22 per cent. positive typhoid agglutination tests in advanced tuberculosis. They also obtained positive typhoid agglutination tests in 15 per cent. of patients with various diseases not typhoid or tuberculosis. Kreuecker (77) also reported positive typhoid agglutination tests in 8 out of 26 cases of tuberculosis (30 per cent.). He believes that such positive findings are common in latent and manifest tuberculosis without typhoid antecedents. All of the positive reactions were in patients with fever. On the other hand, Roth (110) obtained positive typhoid agglutination tests in only 5 out of 100 tuberculous patients at Zurich, and only one with an agglutinating power higher than one in fifty dilution. Such discrepant findings are probably due to differences in the agglutinating capabilities of the strains of typhoid bacilli used for non-specific sera. As has been suggested by Roth, some of the tuberculous patients whose sera gave positive tests may have had earlier unrecognized typhoid and had become chronic carriers.

**PARATYPHOID FEVER**

The organisms now recognized as paratyphosus A and paratyphosus B were first described and studied by Achar and Bensaude (1), and by Widal, and isolated first from the blood by Gwyn (54). It is now conceded that about 3 per cent. of the cases of so-called “clinical typhoid fever” are due to paratyphoid organisms. According to the experience of Johns Hopkins Hospital 2 per cent. of apparent typhoid cases did not give agglutination with B. typhosus, while in Schottmüller’s (118) study of 69 cases of apparent typhoid fever 4 per cent. were paratyphoid. The type of infection produced by these organisms resembles a mild attack of typhoid fever. Conradi (31), in a study of 250 cases supposed to be typhoid fever, found 29 to be paratyphoid fever. Paratyphosus A has the following characteristics: It more clearly resembles the typhoid bacillus in its behavior in milk cultures, in which it does not produce alkali, and leaves the casein undissolved (Jordan). It is less pathogenic for animals than type B, in which it again resembles the typhoid bacillus. Infections in man closely resemble typhoid fever.

B. paratyphosus B has been considered by some observers to be identical with the bacillus of mouse typhoid and of certain strains of B. enteritidis. According to Jordan, type B is probably more widely distributed, and is present in a majority of cases of paratyphoid fever.

Proescher and Roddy (102), however, regard infection with paratyphoid A as more common in America, and Hoskins (60) has reported an epidemic of 35 cases due to this organism. Most authors regard the type B as the more common cause of paratyphoid fever. In many instances infection has seemed to follow the ingestion of infected meat. The organisms are widely prevalent in nature, and are able to lead a sapro-
phytic existence in man and animals from which external contamination of foodstuffs naturally follows. Schmidt (117) and Conradi (loc. cit.) have recovered paratyphus B from the feces of swine, while Ruediger (111) has isolated this organism from the feces of a dog. It has also been found in the feces of the domestic cat (Hunt). The subject has been recently studied by Meinertz (92), who believes, in common with the experience of the Germans, that infection is most frequently derived from meat, especially pork. As a matter of fact, any article of food may contain the organism, such as ice cream, confectionery, oysters, public water supplies, and milk. In most instances the infection reaches the food through human sources as in typhoid fever. The latter disease does not occur in animals, while paratyphoid organisms are more or less commonly found in the intestinal discharges of certain animals, and as such find their way to public water supplies. There are, however, no reasons for supposing that these organisms are usually present in spoiled meat together with putrefactive bacteria. So-called ptomaine poisoning may be due to the ingestion of meat containing the poisonous alkaloids of putrefactive bacteria alone.

Infection with type B seems to be more common in the Middle West than infection with type A. During the past ten years I do not remember having seen an infection due to type A. The diagnosis can, as a rule, only be made by serologic tests of the two types, A and B.

Hunt (64), in a study of four water-borne epidemics of paratyphoid fever, including 509 cases, believes that the disease may occur under one of four general types.

1. That type resembling typhoid fever, most often due to paratyphus A.

2. That type resembling influenza of the abdominal type, due to paratyphus A or B or mixed infection with these and B. typhosus, B. paracoli, or B. alcaligenes.

3. That type with marked gastro-enteric symptoms, such as nausea and vomiting, often diagnosed as ptomaine poisoning.

4. That type resembling dysentery, in which B. dysenteriae is not found, but due to B. enteritidis or paratyphus B. This form is often diagnosed as cholera morbus or ptomaine poisoning.

Saequepee and Bellot (112) were able to obtain type B from the blood of seven out of eight cases of paratyphoid fever. The same organism was obtained from the stools in ten cases, and from the urine in two. The agglutination tests were positive with type B in dilutions of serum varying from 1-350 to 1-900, but were negative with type A and B. typhosus in dilutions of serum of 1-30 and 1-50. These authors were able to trace the

1 Influenza as a clinical entity involving the organs of the abdominal cavity does not come within the range of my experience. Such infections, unless carefully studied and verified by bacteriologic methods, had much better be classified as acute infections of undetermined origin.
infection to a cook, who was responsible for nineteen cases, and who was eliminating type B in the stools.

A short résumé of the symptoms of paratyphoid fever may not be out of place. The incubation period is not definitely known. According to Hunt (loc. cit.) there is a shorter prodromal period and more abrupt onset than in typhoid fever. The study of the agglutination curves made by him in 76 cases of typhoid and paratyphoid fever shows that positive diagnostic reactions were obtained upon corresponding days in relation to the onset of the disease. This would seem to indicate an incubation period of practically the same length. The initial symptoms are apt to be more severe than typhoid in my experience, and are more frequently characterized by nausea and vomiting. The initial rise of fever is apt to be higher than in typhoid, but gradually subsides in 5 or 6 days and disappears by lysis in 12 to 15 days. The morning remissions may be marked. Bronchitis may be present, and a few rose spots are usually found early in the disease. The spleen is generally enlarged. Constipation is more frequently present than diarrhea, although diarrhea may occur in the forms characterized by symptoms of gastro-enteritis during the incubation stage. Paratyphoid organisms are frequently found in the blood stream during the first week. It has been stated by some authors that the form characterized by gastro-enteritis does not show the infection in the blood stream so frequently. The infection of the solitary lymph follicles and Peyer's patches usually leads to more or less ulceration, the type of which resembles more closely that seen in dysentery than in typhoid. The ulcerations are, as a rule, not deep, and the edges are not undermined. Hemorrhage, which may occur from the erosion of a small blood vessel, is usually slight and seldom extensive. Perforation of an ulcer-bearing area in paratyphoid fever has not been reported to my knowledge. The case mortality is considerably lower than in typhoid fever. It probably does not generally exceed 2 to 3 per cent., although in two epidemics of 43 cases due to paratyphosus B studied by Hunt the mortality was 14 per cent. The paratyphoid bacillus may be found in periosteal abscesses or in abscesses of bone, occurring as a complication in typhoid or paratyphoid fever. The organisms have been isolated from cases of orchitis, cystitis, and pyelonephritis, from a case of thyroid abscess by Widal, and from a bone sinus following an old abscess by Jensen and Kock (67). They have also been isolated on necropsy from the liver, spleen, and kidneys of individuals who had succumbed to the infection.

EXPERIMENTAL AND CLINICAL DATA CONCERNING THE USE OF TYPHOID VACCINES

Experimental work upon animals has seemed to show that following the injection of typhoid vaccine the agglutinins and opsonins of the blood
serum are increased. This has also been found to occur in man following antityphoid inoculation. The agglutinins and opsonins are especially increased between the eighth and thirtieth days following the inoculation. There are few evidences of antibody production during the first week. In addition, according to Albert and Mendenhall (4), the injection of typhoid vaccine causes a marked absolute and relative large mononuclear leukocytosis. In fact, this is the only leukocytic change common to typhoid fever and antityphoid inoculation. Weston (138) has shown that in individuals supposedly in good health the blood serum has universally failed, in his experience, to cause agglutination in dilution 1-250. Ten days after vaccine inoculation the blood serum of such individuals gave the following agglutinative results:

- Positive in dilution 1-250, 100 per cent.
- Positive in dilution 1-500, 97.7 per cent.
- Positive in dilution 1-1,000, 92.2 per cent.

In 74 instances the serum agglutinated in dilution of 1-10,000.

In a series of 24 persons studied by Wollstein (140), in which typhoid vaccine was used, it was found that the apparent highest point of immunity was reached within one month after the last inoculation, and that the presence of immune bodies rapidly diminished during the subsequent two months. In 8 of 19 cases, studied ten months after inoculation, bactericidal substances were absent, while 15 of the 19 were negative after thirteen months. She found only one serum capable of reacting in dilution 1-200 at the end of thirteen months. The clinical immunity apparently lasts much longer than these figures indicate.

The experimental studies mentioned have dealt with individuals in health. It remains to be seen what variations are applicable in the presence of disease. Sappington (114) and others have described the opsonic index curve during typhoid, which averages about 2 during the early part of the disease. It is decreased slightly during the course of high fever, and rises again during the decline and convalescence. As a rule, it reaches normal soon after recovery. The injection of vaccines does not greatly influence the course of the index one way or the other. It has for a number of years been realized that the opsonic curve could not be relied upon as an index for treatment by vaccines. In typhoid some patients who are in extremis from asthenia will show a persistently high index, while those who are progressing favorably toward recovery may show a low index. As a rule, however, the opsonic curve follows the clinical course of the disease, being low when the patient is very ill and high when improvement is taking place. In the production of experimental typhoid in animals Metchnikoff and Besredka (93) have shown that vaccination with killed bacilli protects guinea-pigs against peritoneal infection, but that it was powerless
to prevent the disease in apes. The apes were the only animals susceptible to typhoid infection by way of the mouth. This shows, these authors believe, that a mistake is made in attempting to apply to man the results of experimental research in guinea-pigs and rabbits.

Bruscettini and Paccanaro (23) have found that guinea-pigs resisted otherwise fatal doses of typhoid toxin after a preventive injection of vaccine to which had been added one-tenth part of a leukocyte extract. They obtained the leukocytes by injecting an irritating substance into the pleural cavity of other animals. The vaccinated animals showed no signs of the disease fifty days later, although all control animals died in the first 48 hours.

Pescarolo and Quadrone (99) are exponents of the method of injection of living typhoid bacilli in the production of immunity. They contend that such a procedure is entirely harmless and causes only transitory symptoms of a local nature. They believe it to be of especial value in the treatment of generalized infection due to the typhoid bacillus.

Broughton-Alcock (22) has sensitized living typhoid bacilli after the method of Besredka by adding a small quantity of antityphoid horse serum for 24 hours. The bacterial cells were then centrifugated and suspended in salt solution. The bacilli remain alive for over four months. The living vaccine is used in doses of 500 to 1,500,000,000. He states that there is no general, and only a slight, insignificant local reaction following the injections. He believes the production of immunity to be ideal by the living vaccine, and that the method is preferable to the use of killed bacilli. Over 750 persons have been inoculated by this method, and the results were satisfactory and encouraging.

Castellani (26) has also been an advocate of the method of using live attenuated vaccine according to the method introduced by him in 1904. He believes the method harmless and productive of higher immunity than can be obtained through the use of killed vaccine.

Bassenge (13) believes that a 1 per cent. emulsion of lecithin in sterile water has the property of dissolving typhoid bacilli, and that this emulsion, when introduced into the peritoneal cavity of guinea-pigs, protects them from subsequent intraperitoneal injection of lethal doses of the bacilli, provided a period of 24 hours had elapsed after the lecithin solution was injected.

Johnson (69) has shown that the condition analogous to the typhoid carrier state can be produced in rabbits. The bacilli could be recovered from the blood, feces, and gall-bladder of 9 out of 11 unvaccinated animals, but were not obtained after inoculation from 5 vaccinated animals.

Uhlenhuth and Messerschmidt (134) were able to secure similar results in rabbits. By direct inoculation of the gall-bladder they succeeded in producing chronic bacilli carriers. After six months the bacilli were still present in the gall-bladders. By previous vaccination they found it
impossible, however, to prevent the growth of bacilli in the gall-bladders of the animals. They found in addition that the carrier state could not be cured by subsequent vaccination.

**THE BACTERIAL THERAPY OF TYPHOID AND PARATYPHOID FEVERS**

The preceding divisions of this chapter have covered the essential bacteriologic and serologic factors necessary to rational diagnosis in the consideration of infection by members of the so-called enteritidis group. These facts are essential, since rational bacterial therapy can only be successful, if successful at all, when based upon an adequate foundation of bacteriologic knowledge. Since the earlier days of bacterial therapy the impression has been gaining ground that treatment by bacterial vaccines was of no avail, if not positively harmful, in any of the septicemic and bacteriemic infections. Many seemingly fortunate adaptations of this form of treatment have been recorded in such conditions. The question is by no means settled whether many of the recoveries in such instances have not occurred in spite of the treatment rather than because of it. The optimism so frequently associated with any innovation in treatment is apt to be replaced by an increasing pessimism unless universal experience sanctions the method. This should not be interpreted as a belittlement of interest in the subject, but rather should be regarded, in the light of critical analysis, as a disposition to tally uncertain experiences among the uncertainties.

It has been with considerable interest, therefore, that the attempt has been made to analyze carefully the reports occurring in the literature of this subject during the past five years. The most complete analysis of the bacterial therapy of typhoid fever has been made by Watters (136), who has summarized the results obtained in the treatment of 1,120 cases, of which 158 had been treated by himself during the past six years. If ten deaths are excluded in his series, because he believes the patients to have been *in extremis* when the treatment was begun, the mortality in the remaining 148 cases was 4.7 per cent. If the ten deaths were not excluded the mortality was 11 per cent. in the treated cases. He states that during a similar period of observation in 100 patients with typhoid not receiving vaccines there was a mortality of 13 per cent. There appears to be some reason why patients who are *in extremis* upon admission, and those receiving only one inoculation, should be excluded in the consideration of statistics, but that is about as far as exclusions should go. In brief, it appears from Watters’ series that (1) the mortality was slightly lower among the patients treated by vaccines than among those who did not receive such treatment; (2) the duration of fever was about ten days less in
the treated cases, and (3) the percentage of relapse was diminished in the treated cases.

From Watters' analysis of 1,120 cases treated by bacterial therapy the following results were obtained by 9 other observers who had each treated more than 25 cases:

<table>
<thead>
<tr>
<th>Reporter</th>
<th>Number of cases</th>
<th>Relapse</th>
<th>Death</th>
<th>Mortality per cent.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semple (119)</td>
<td>60</td>
<td>2</td>
<td>2</td>
<td>3.3</td>
</tr>
<tr>
<td>Sadler (113)</td>
<td>92</td>
<td></td>
<td>14</td>
<td>15.2</td>
</tr>
<tr>
<td>Hollis</td>
<td>51</td>
<td>8</td>
<td>2</td>
<td>3.9</td>
</tr>
<tr>
<td>Meakins and Foster (91)</td>
<td>41</td>
<td>1</td>
<td>1</td>
<td>2.4</td>
</tr>
<tr>
<td>Smallman</td>
<td>36</td>
<td>0</td>
<td>3</td>
<td>8.3</td>
</tr>
<tr>
<td>Callison (25)</td>
<td>38</td>
<td>1</td>
<td>5</td>
<td>13.1</td>
</tr>
<tr>
<td>Richardson (105, 106)</td>
<td>28</td>
<td>1</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Sanborn</td>
<td>35</td>
<td></td>
<td>4</td>
<td>11.4</td>
</tr>
<tr>
<td>Gray (51)</td>
<td>128</td>
<td></td>
<td>5</td>
<td>4.0</td>
</tr>
<tr>
<td>Total number of cases</td>
<td>507</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average mortality</td>
<td></td>
<td></td>
<td></td>
<td>6.8</td>
</tr>
</tbody>
</table>

The average mortality in this series of 507 cases is thus seen to be somewhat lower than the usual mortality among patients not treated by bacterial therapy. The average mortality is, however, very difficult to determine, since variations in the virulence of the infection and in the type of resistance encountered may affect the rate. It has been recognized for years that the mortality varies in different years and in different epidemics. In the figures given above considerable variation occurs. Thus the mortality was zero in Richardson's series of 28 cases, while it was 15.2 per cent. in Sadler's series of 92 cases. Nearly all observers have mentioned that relapses are less frequently encountered when typhoid patients are treated by bacterial vaccines. Watters' comparative series of 89 patients treated with vaccine showed relapse in 4.9 per cent., while 87 patients treated in the same institutions without vaccines showed relapse in 20 per cent. It is somewhat surprising that the dosage of vaccine used should have so little definite effect upon the fever curve, the leukocyte count, or upon the mortality rate in the hands of different observers. For example, Semple (loc. cit.) used doses of from 50- to 200,000,000 in 60 cases and had a mortality rate of 3.3 per cent. Smallman used doses of 100- to 300,000,000 in 36 cases and had a mortality rate of 8.3 per cent., while Meakins and Foster (loc. cit.) used doses of 1,000- to 2,000,000,000 with a mortality rate among 41 cases of 2.4 per cent. Hollis used doses of 10- to 250,000,000 in 51 cases with a mortality rate of 3.90 per cent., and Callison used doses of 500,000,000 in 38 cases with a mortality rate of 13.1 per cent. Most of these authors have felt convinced that patients
treated with vaccines suffered less depression, had lower temperatures, were brighter, and had fewer complications. The opinion seems to be that, conservatively used, vaccines can do no harm in the disease.

The question of possible beneficial effects to be secured by vaccine treatment as heretofore applied will now be discussed. It must be confessed that as yet the accumulated statistics are not convincing. Nor can beneficial effects be expected in generalized infections as heretofore applied if the conception of the disease founded upon the principle of poisonous split products of protein disintegration is proved and accepted. In fact, almost from the beginning of bacterial therapy the belief has been repeatedly expressed that little could be expected of bacterial vaccines in generalized infections. Many clinicians have, however, applied vaccine therapy in the treatment of such conditions with apparent good effect. The belief is, however, gaining ground that many such recoveries would have taken place without vaccine treatment, and that such benefits as seemed to follow were incidental rather than essential to the recovery. As a general principle it would appear unreasonable to continue to add fuel to the flames. The body cells are, so far as present knowledge takes us, busily engaged in eliminating the poisonous protein groups resulting from the death of the bacterial cells in considerable quantity in the body. In most instances their efforts are successful, and recovery takes place. To thwart their efforts by adding more bacterial protein, when already worked to capacity, would appear unjustifiable. It does not appear that the body cells require in any uncomplicated infection such an added stimulus to combat the bacterial invaders present in enormous numbers in any bacteriemia. The stimulus to antibody formation already exists in such conditions, and when sufficient time has elapsed, as measured by the incubation period of the disease, the cells have acquired specific properties not previously possessed, making possible the destruction of the bacteria. If this condition did not exist the continued unrestricted development of bacteria in the blood stream would so impair the functional capacity of all body cells as to lead to tissue death.

The intravenous administration of the ordinary nonsensitized vaccine is certainly not without an element of danger and is not to be recommended.

Sensitized Vaccine Therapy.—The recent adoption by many clinicians of the sensitized vaccine of Besredka ¹ carries with it much more rational belief that such a product will prove beneficial than those heretofore used. The killed organisms, according to Besredka, when exposed to the action of immune serum become saturated with specific antibodies present in the serum, and are thus ready for immediate phagocytosis and digestion by complement when injected into the body of the patient. In the preparation of the vaccine the serum is removed by centrifugation after the bac-

terial cells have become sensitized. This preparation produces but slight local or general reaction and may be given in larger doses and more frequently than the unsensitized vaccine. The immunizing effect, according to Besredka, becomes manifest within 48 hours.

In those types of typhoid infection mentioned above the use of sensitized killed vaccine appears to offer more rational hope of the accomplishment of active immunity than could possibly be secured by unsensitized vaccines heretofore used. In other words, the hope appears to be justified, from the work so far done, that the disease process may be abruptly shortened through the use of sensitized vaccine if used early in the course of the fever. Gay and Chickering (49a) have recently reported the most encouraging results so far obtained. They have used a sensitized polyvalent killed vaccine sediment prepared after the method of Gay and Claypole. The dosage used by them varied from 1/50 to 1/25 mg. of the dried sensitized vaccine intravenously, which they have found corresponded to a dose of 150 to 300 million bacteria. In sixty-five cases a distinct benefit was obtained in 66 per cent., in over half of whom the recovery was of an abortive type with a critical fall of temperature and the establishment within a few days of a permanent normal temperature. This permanent normal temperature was reached on an average seven days after beginning treatment. The sensitized vaccine sediment produces but slight reaction as compared with the nonsensitized vaccines heretofore used intravenously. The dose may be repeated every two or three days, depending upon the clinical signs of reaction. The vaccine probably acts as a marked stimulant to phagocytosis. A series of subcutaneous injections is recommended by Gay and Chickering following the intravenous treatment as an aid in the prevention of relapses.

**The Vaccine Treatment of Complications.**—In localized infections incident to convalescence, such as periostitis, glandular suppuration, cholecystitis with rupture or drainage and infection of the drainage tract, appendiceal and intestinal perforation necessitating drainage, mastoiditis and cystitis due to B. typhosus or secondarily infecting organisms, an autogenous vaccine is of great service. Good results may be expected from a stock typhoid vaccine if an autogenous vaccine is not obtainable. Bacteriologic examinations should be made of the material from the infected area to determine the causative organisms. In typhoid peristitis, which may appear during convalescence or a number of years subsequently, the staphylococcus albus or aureus is frequently encountered in addition to the B. typhosus, while in abdominal complications involving the gall-bladder, the rupture of mesenteric suppurating glands, appendicitis, or cystitis, the colon bacillus is apt to be present.

A corresponding vaccine should be used in such conditions. The appropriate dosage of these organisms, judging from the signs of local and general reaction, has been found to be as follows: of B. typhosus 100- to
300,000,000, of staphylococci 200- to 500,000,000, and of B. coli 50- to 100,000,000.

The Typhoid Carrier and Vaccine Treatment.—Park (98), in 1908, estimated that "probably one in every five hundred adults who has never knowingly had typhoid fever is a typhoid bacillus carrier." Such individuals may be termed primary, idiopathic, or contact carriers. They become such, as a rule, through association with those who have the disease. Their own tolerance to the infection is probably due to natural immunity or to a partial immunity from an earlier unrecognized mild typhoid infection. Instances are common among hospital attendants and nurses. Such persons may continue to eliminate the bacilli in the urine for years, but a majority disseminate the infection with the feces. As a rule, such individuals experience little, if any, inconvenience. The attention of the physician is attracted by the apparent connection of such persons handling food products with endemic outbreaks or by vague symptoms which may lead to an investigation.

Houston (61) in 1899 reported the first case of typhoid bacilluria in a contact carrier who for three years had shown symptoms of chronic cystitis. Drigalski and Conradi (quoted by Park) in 1902 found typhoid bacilli in the stools of four persons who had had no previous typhoid symptoms, but who had been in contact with typhoid fever. Since then many similar cases have been reported. In the diagnosis of a suspected contact carrier the history of exposure to the disease is important. The agglutination reaction is increased in at least 45 per cent. of the cases, and the opsonic index to the B. typhosus is much increased. Hamilton (56) found that an abnormally high index was present in all of the seven cases examined by her. All of her patients had manifested symptoms of gall-bladder disease.

In estimating the opsonic index for B. typhosus the serum of the suspect and control should be heated to 58° C. for fifteen minutes. In this way the lysin for typhoid bacilli, according to Hamilton, is destroyed, while the heat-resisting element of the opsonin remains, making a decided contrast between normal serum, which contains little of this element, and the immune serum of a carrier which is usually rich in it. Gaechtgens (48) found an unusually high opsonic index which averaged 2.8 in 16 typhoid carriers. Gould (50) also found that the opsonic index was increased, and that the percentage of large mononuclear leukocytes was increased in typhoid carriers. The bacilli should also be sought for in the stools and urine.

Since primary or contact carriers, as well as secondary carriers following convalescence from typhoid, possess a partial immunity to the infection, it may be considered, judging from their increased opsonic index and agglutinative powers, that their immunity is phagocytic, not bacteriolytic or bactericidal.
Typhoid vaccine has been shown to bring about the production of an efficient immunity when used for prophylactic purposes. In order to prevent those in association with typhoid patients from becoming carriers it follows that all those brought in contact with the disease, such as members of the families in which typhoid occurs, nurses, orderlies, and physicians, should receive immunizing treatment. Many hospitals require such immunization of all nurses and orderlies upon taking up their residence. In this way the immunity acquired not only protects them against the infection, which possibly may culminate in an attack of the disease, but prevents in a large measure the perpetuation of the bacilli within their bodies and the development of the carrier state. The immunizing doses generally used consist of a first injection of 500,000,000, followed at weekly intervals by two injections of 1,000,000,000 each. The dosage should depend somewhat upon the age of the patient, the general resistance, and the local and general reaction. These doses have been used in the immunization of nurses at Flower Hospital. Although an occasional reaction could be characterized as severe, the majority were mild. We have used a combination typhoid and paratyphoid vaccine in the proportion of three strains of the former to two of the latter.

Richardson and Spooner (106), in the immunization of nurses at Massachusetts General Hospital, have used four injections, at five-day intervals, of from 50- to 400,000,000.

Gaehhtgens (loc. cit.) found in a series of 386 cases of typhoid fever that 77, or 20 per cent., could be traced with great probability to chronic typhoid carriers. Park (loc. cit.), of the New York Health Department, found bacilli in the stools in 6 per cent. of the cases examined, 8 to 10 months after convalescence, and in 5 per cent. of the cases just as they left the hospitals after typhoid. It is variously estimated that from 2 to 5 per cent. of all convalescents become so-called chronic secondary carriers. Fütterer (47) in 1888 first called attention to the presence of typhoid bacilli in the gall-bladder. Since then a large number of observations have confirmed the fact that the infection may be perpetuated in the gall-bladder for many years following convalescence. The bacilli are intermittently ejected and excreted in the feces. About four times as many women as men become chronic carriers, according to Conradi (loc. cit.). A smaller proportion of carriers excrete the bacilli in the urine. Kaspar (73) was able to cultivate typhoid bacilli from the bile removed from the stomach after a test oil breakfast seven years subsequent to an attack of typhoid. In many instances no symptoms pointing to cholecystitis are present.

Epidemics have been frequently traced to typhoid carriers. In many instances the infection has been spread by cooks, waiters, workers in confectionery and ice cream, hucksters, dairy maids, and individuals who handle food products. In Huggenberg’s report (62) an epidemic of 13 cases was traced to a woman who had typhoid 31 years previously, and
who continued to eliminate typhoid bacilli in the feces. In Bernhuber's report (15) 16 cases were traced to a cook who had her attack of typhoid 22 years before. Gregg (52) has reported the case of a boarding-house mistress who was apparently the source of infection in 7 cases of typhoid 52 years after her own recovery.

Currie and McKeon (36) have traced the infection of 28 men to a typhoid carrier who continued to eliminate bacilli in the stools 4 years after his attack, while Crumbine (34) has reported a most unusual instance in which it was estimated that 76 cases of typhoid originated primarily or secondarily from a typhoid carrier with a permanent biliary fistula following an operation for gall-stones. Her attack of typhoid had occurred 9 years previously. Jundell (72) has described the conditions present in a family, the grandmother of which was known to be a typhoid carrier for 54 years. During the interval 22 members of the family were attacked. This carrier was 83 years old, and her feces were found to contain typhoid bacilli before death. Upon autopsy the gall-bladder was found to be normal, but pure cultures of typhoid bacilli were obtained from it. The blood and urine were sterile. Hutchinson (65) has reported seven instances of typhoid traceable to a carrier. The period of infectivity began about 5 years after her attack and covered a period of 10 years.

The treatment of the acute typhoid carrier, shortly after convalescence from the disease, by so-called intestinal and urinary antiseptics and cholagogue cathartics, has been found to give much more satisfactory results than the same treatment of the chronic carrier. Such results would be expected since, during convalescence, the natural immune bodies are increased and the additional help secured by medicinal means suffices in many instances to eradicate the infection. In chronic carriers the bactericidal and bacteriolytic properties of the blood serum are decreased, and such medicinal assistance as may be offered is of little avail.

Liefmann (82) has claimed that disappearance of the bacilli from the stools of three carriers followed a Bulgarian sour-milk diet maintained for a number of months. Leary (80) and others have recommended the removal of the gall-bladder in chronic typhoid carriers in which medicinal and vaccine treatment had failed. In Leary's two patients the bacilli disappeared within 90 days after cholecystectomy. On the other hand, Loele (78) has described a carrier operated upon for cholelithiasis three months after an attack of typhoid. The bacilli had been constantly present in the stools, but none were found in the gall-bladder at the time of operation. In Holmes' (New York Med. Jour., Aug. 12, 1911) patient the bacilli disappeared from bile and stools in 8 weeks after cholecystectomy.

The number of chronic carriers successfully treated by vaccines has not been large. Irwin and Houston (66) were able to cause disappearance of the infection in a patient who for 7 years had been a carrier. She
received during two months five injections, varying from 50- to 500,000,000. Meader (90) has also successfully treated a carrier and found that the best therapeutic dose varied between 75- and 400,000,000. He has shown that the disappearance of the infection coincided with the increase in the bactericidal power of the patient’s blood serum. Brem and Watson (20) have reported the apparent cure of a child whose urine was found to contain bacilli a few months after convalescence. She received nine injections of autogenous vaccine in about two months, when the urine was found to be free from bacilli. The agglutinative powers of the patient’s serum increased to 1 in 1,000 during the course of the inoculations.

In 1910 I reported (127) the apparent cure of a typhoid carrier by means of an autogenous vaccine. This patient began to suffer vague abdominal distress with tenderness over the gall-bladder and occasional painful micturition about one year after an attack of typhoid fever. Cultures from the urine showed two types of organisms; both were motile. Neither liquefied gelatin, but one, the preponderating type, corresponded to the B. typhosus. This organism did not produce indol or acid in litmus gelatin, nor coagulate milk, nor produce gas in glucose gelatin. The other organism corresponded to the colon group. The typhoid opsonic index was 2.3 and the patient’s blood serum produced a positive agglutinative reaction in dilution 1-20 in one-half hour with a stock typhoid culture, and in dilution 1-40 with the organisms isolated from the urine. The patient received six injections of an autogenous vaccine at intervals of about 12 days in doses varying between 100- and 400,000,000, after which no more organisms could be found in several specimens of urine. Her blood serum one month after the series of inoculations would agglutinate a stock typhoid culture in dilution 1-100 and her subjective symptoms, such as tenderness over the gall-bladder and painful micturition, disappeared. The bactericidal and bacteriolytic properties of her blood serum were also increased by the inoculations.

In 1912 I described the apparently successful treatment of another carrier 7 years after his attack of typhoid fever. The following details of the history and treatment are taken from the American Journal of the Medical Sciences for April, 1912:

R. F., aged twenty-eight years, was referred January 17, 1911, as a possible typhoid carrier, although no cases of the disease were traceable to him. In 1904 this patient had typhoid fever for six weeks with apparent recovery for one year, after which time he began to complain of pain in the region of the umbilicus, which continued until 1909. In 1909 diarrhea began, the bowel discharges numbered five or six daily, with some mucus, and in December, 1909, three severe hemorrhages from the bowel occurred with temperature as high as 104° F. The last hemorrhage measured over 1 quart. The diarrhea continued for one year thereafter. The discharges were occasionally streaked with blood and mucus and gradually subsided under medication. The weight before the attack of typhoid
seven years ago was 151. At the time of examination it was 134. He complained
of frequent headaches. The examination revealed nothing of importance on the
part of the heart, lungs, nervous system and abdomen, except that some tenderness
upon pressure was present below the umbilicus. One loose stool occurred every
morning, with poor control of the sphincter ani. Examination of the fresh stool
for amebae dysenteriae, as well as stains of the mucus present for tubercle bacilli,
were negative. The von Pirquet skin reaction was negative. The leukocytes num-
bered 7,200, and the urine was negative to albumin, pus and sugar. There had
never been any urinary symptoms. The Adler occult blood test was positive in
the stools. Cultures taken from the stools showed the presence of the colon bacillus
and a short, plump, actively motile rod, which in pure culture did not produce
acid in litmus gelatin, or gas in stab culture on glucose agar, nor was indol pro-
duced after ten days' growth in bouillon. Milk was not coagulated. The agar
slant showed a growth which resembled that of the typhoid bacillus, while the
growth was invisible on potato. Gelatin was not liquefied in six weeks. Cultures
from the urine showed a few isolated colonies of staphylococcus albus (probably
contamination). Subsequent cultural studies of the fresh stool gave the same
findings. His blood serum would agglutinate a stock typhoid culture in dilution
1 to 20, while the autogenous strain would agglutinate in dilution 1 to 10.

A vaccine was prepared from the typhoid organisms present in the stools. He
received his first inoculation on February 6, 1911. Between that date and May 5,
1911, 14 inoculations were given at weekly intervals, the dose varying between
110,000,000 and 510,000,000. Negative cultures were obtained in May, 1911.
Since that time he has received five additional inoculations, three of which have
contained 1,000,000,000. The agglutinating power of the patient's serum was in-
creased ten-fold during the course of treatment. Coincident with the vaccine treat-
ment and the increase in the agglutinative powers of his serum his general con-
dition improved. The tendency to loose stools with poor sphincter control dis-
appeared, the bowel discharges became normal in appearance, gave negative cul-
tures, and he regained his normal weight.

Since typhoid carriers are able to transmit the disease through hand-
ling food supplies, food and milk inspection should be rigidly carried out
during epidemics. Individuals recently ill with the disease should be
forbidden the handling of food products for at least one month following
their recovery. This should include workers in confectionery, waiters,
cooks, dairymen and maids, bakers, butchers, and hucksters. Such indi-
viduals should be properly instructed in methods of personal cleanliness
and should be obliged to scrub their hands with a clean brush, soap and
water for at least five minutes after each visit to the toilet before they
are allowed to handle food products. If municipalities were to refuse to
license any milk handler or any individual handling food who could be
shown to be responsible for the spread of typhoid and paratyphoid fever,
it would be a step of great prophylactic importance. Many carriers of
these two infections exist. It should be within the police powers of any
municipal board of health to investigate bacteriologically any suspected
carrier engaged in handling food products. This would be particularly
important for the apprehension of convalescents who begin work while
still capable of transmitting infection. If such individuals become chronic
THE BACTERIAL THERAPY

Carriers they should be furnished employment which does not have to do with handling food and over which the authorities could exercise some supervision.

In brief it may be stated that the treatment of carriers by vaccine inoculations offers more hope for cure than any other known method. Not all will be cured, but the bacilli will disappear in some instances. When typhoid bacilli have produced clinical evidences of cholecystitis, the symptoms of which do not as a rule appear until obstruction of the cystic or common duct is present, it is doubtful whether vaccine treatment will of itself be of avail. Surgical drainage of the viscus will then be necessary. On the other hand, appropriate vaccine treatment should be tried in individuals showing the bacilli in stools or urine subsequent to contact or subsequent to the disease without cholecystitis. The method of dosage found to be most satisfactory has been to increase gradually from 100- to 1,000,000,000 at 7 to 10 day intervals, depending upon evidences of immunizing response as obtained by serologic and culture studies.

Preparation of the Vaccine.—Pure cultures of stock or autogenous organisms are grown on agar for 24 hours, when the growth is washed off with sterile salt solution. The resulting suspension is thoroughly shaken to break up the clumps and a small quantity drawn into an ordinary red blood pipette, diluting 1-200 with salt solution for purpose of standardization. A small drop of this dilution is then placed upon the blood-platelet counter of Helber-Zeiss. The blood-platelet counter differs from the ordinary red cell counter in that with the former the depth of the chamber is only 1/50 mm. Twenty-five small fields are counted under the microscope, using dim direct illumination or indirect illumination by the dark-field condenser, and the sum divided by twenty-five to obtain the unit value. The unit value is then multiplied by the dilution 200, by the depth of the chamber 50, by 400, the number of small squares in a millimeter, and, lastly, by 1,000 to convert to cubic centimeters. For example, if fifty bacterial cells are counted in twenty-five small squares the unit value per square would be 2, multiplied by 200, by 50, by 400, and by 1,000, or 8,000,000,000, the number of bacteria per c. c. of suspension. The suspension is then heated to 54° to 55° C. for one hour in a test tube in a water bath, and then diluted with sterile salt solution containing 0.35 per cent. phenol, so that each cubic centimeter of the finished vaccine contains approximately 1,000,000,000 bacteria. Care must be exercised not to overheat the vaccine, since overheating impairs its immunizing properties. The thermal death point of most strains of typhoid bacilli varies between 53° and 56° C. Cultures should be taken from the vaccine to insure sterility before using.
SERUM THERAPY

Chantemesse (27), of Paris, has for years advocated serum therapy in typhoid fever as an adjuvant to the well-established principles of treatment of this disease. His recent figures (1913) show a mortality of 4.3 per cent. among those who received the serum treatment, while the mortality was 17 per cent. among those who did not receive it. These figures cover several thousand cases in the same city over the same period of years. There can be little doubt that animals in health can be actively immunized to the living typhoid bacillus, and that their immune serum possesses marked agglutinative and bactericidal power. It has, however, been found difficult to transfer such active immunity present in the animal to the patient. Such serum is not, in a strict sense, an antitoxic, as in diphtheria and tetanus, since it is not a question in typhoid of neutralization of toxins elaborated by living bacteria in the body. As an antiserum, according to Chantemesse, it causes the destruction of great numbers of bacteria in the body through its agglutinative and bactericidal powers. There is no reason, however, for believing that the body cells in the ordinary uncomplicated case of typhoid fever are not busily engaged in the destruction of bacteria, nor are there reasons for believing that the body cells can be stimulated to further activity when worked to their full capacity.

If the conception of Vaughan and his pupils is accepted, the manifestations of disease, such as fever, prostration, and emaciation, are due to poisonous split products of bacterial protein disintegration. The symptoms are present as long as there are bacteria to be destroyed in quantities sufficient to produce protein poisoning. If the body cells in an individual of lowered resistive powers were unable to cope with the infection and unable to destroy more bacteria each day during the disease than were being reproduced, the course of the disease would be prolonged indefinitely until death would occur from exhaustion. Such apparently occurs occasionally in the course of typhoid fever. Ten years ago deaths from exhaustion were much more commonly seen than to-day, due largely to differences in feeding methods. It is exceptional now to see uncomplicated 45- and 50-day fever cases, more or less common a decade ago. In such instances of prolonged fever an antiserum possessing increased agglutinative and bactericidal properties may be of value to stimulate dormant body cells to greater activity than they have previously possessed, and thus shorten the disease. For that matter, the injection of normal human serum seems to be of assistance in any prolonged overwhelming infection. The serum can be conveniently obtained under aseptic precautions by venesection from some healthy relative, and stored in the ice-chest. The injection of 10 c. c. intravenously or 20 c. c. subcutaneously every four hours for 10 or 12 doses has in my hands seemed to turn the tide toward recovery in several
REFERENCES

desperate cases. The majority of clinicians have not been convinced that
typhoid antiserum has any marked effect upon the uncomplicated course
of the disease.

Niecloaee and Conseil (97) have attempted to transfer the active.im-
munity present in convalescent typhoid patients to those ill with the disease
by means of the blood serum. They employed the serum of patients after
the temperature had been normal for from five to eight days. This serum
was injected in repeated doses. The duration of the disease was not
shortened, although certain nervous symptoms seemed to be improved.
They used 685 c. c. of serum furnished by 15 convalescents in 5 pa-
tients with typhoid fever. It is obvious that, aside from its scientific
interest, such a procedure in practice could hardly become general. In
the first place, typhoid convalescents are as a rule sorely in need of all the
blood serum they possess, while, in the second place, the possibility of the
transference of other diseases from patient to patient prohibits its use.

REFERENCES

   1896, xiii, 820.
2. Achard, C., and Flandrin, C. Ann. de méd. et chir. infant., 1911,
   xv, No. 17.
   232.
    J. A. M. A., 1911, lvii, 785.
16. Bigelow, E. B. J. A. M. A., 1911, lvii, 1418; ibid., 1912, lviii,
    1339.
17. Bolduan, C. F., and Noble, W. C. N. Y. Med. Jour., 1911, xciv,
    J. A. M. A., 1908, l, 84.
TYPHOID AND PARATYPHOID FEVERS

27. Chantemesse, A. Gaz. des Hôp.; 1898, lxxi, 397; Presse méd., Paris, 1906, xiv, No. 16; Gaz. méd. de Paris, 1907, x, 2; Le Monde méd., June 15, 1913.
44. Fornet. Münch. med. Woch., 1906, liii, No. 22.
REFERENCES

51. Gray, G. A.  Northwest Medicine, 1913, v, No. 2.
70. Jordan, E. O.  General Bacteriology, 1908, p. 256.
TYPHOID AND PARATYPHOID FEVERS

REFERENCES

    1911, lvi, 1906.
133. Triboulet, H. Arch. de méd. d. enfants, 1909, xii, No. 8.
134. Uhlenhuth and Messerschmidt, T. Deutsch. med. Woch., 1912,
    xxxviii, 2397.
135. Vaughan, V. C., V. C., Jr., and J. W. Protein Split Products,
    1913.
137. —— and Eaton, C. A. Ibid., 1909, lxxv, 93; ibid., 1911, lxxix,
141. Worms, G., and Hamant, A. Arch. gén. d. chir., 1912, vi, No. 2.
CHAPTER VII

BACTERIAL THERAPY IN LESIONS PRODUCED BY THE BACILLUS COLI COMMUNIS

WILLARD J. STONE

BACTERIOLOGIC CONSIDERATIONS

The B. coli communis was discovered by Escherich in 1885. The original culture was obtained from the bowel discharges of a breast-fed infant. This organism has been found widely distributed in nature, and is almost constantly present in the intestinal tract of man and many of the higher animals. It is often found in almost pure culture in the large intestine, but in the small bowel it grows as a rule in association with many other bacteria, the most important of which is the B. lactis aerogenes. The B. coli can be easily cultivated from the stools by any of the ordinary aerobic methods. It has been cultivated from the dejecta of infants in from 4 to 18 hours after birth. It is probably identical with the B. neapolitanus of Emmerich and the B. pyogenes foetidus of Passet.

Because of its widespread distribution in nature the B. coli, or, as it is commonly called, the colon bacillus, may occur as an etiologic cause in a variety of conditions, sometimes as the sole organism present and again in association with harmless saprophytes or with pathogenic varieties. It is one of the strange arrangements of Nature which permits the development of a variety of organisms within the body in harmless contact with certain tissues, while, if transported to other tissues, the cells of which apparently are not sensitized or immune to their presence, their development there leads to tissue destruction. For example, the colon bacillus, while harmless when in contact with the cells of the intestinal mucosa, may produce a fatal peritonitis when developing in contact with the endothelial cells of the intestinal serosa.

There can be no doubt that the pathogenicity of the colon bacillus has been exaggerated. On the other hand, its frequent association with certain septic processes cannot be doubted. In appendiceal abscesses, in cholecystitis and cholangitis, in cystitis and pyelitis, in acute prostatitis, in peritonitis, in septicemia processes with multiple abscesses in soft tissues
or bone, or in septic thrombus following abdominal operations, it is frequently found. There can be no doubt also of the great increase of the colon bacillus in the intestine during typhoid fever as well as during other pathologic ulcerative or obstructive lesions affecting the bowel. In fact, many writers, among them Sanarelli, are disposed to regard some of the pathologic changes ascribed to typhoid to the increased virulence assumed by B. coli in the presence of the typhoid bacillus.

The conditions necessary for the migration of the B. coli from the intestinal tract into the blood stream or into the lymphatics, by means of which the organisms may be transported to more or less distant tissues, are probably intimately connected with trauma and separation of tissue continuity. For example, a rectal fissure, a tuberculous or carcinomatous ulcer, or small thrombi in vessels incident to surgical procedures may serve as the point of entrance. It is probably true that the organisms frequently reach the lymph tributaries to mesenteric glands, where their progress is stopped. Given, however, a temporarily lowered resistance, the organisms may overcome cellular activity in the glands and, reaching the blood stream, be carried to other tissues. In this way may be explained the suddenness of onset of certain attacks of cystitis and prostatitis, following cold and exposure.

The lesions produced in animals by injection of B. coli are very similar to those produced by the B. typhosus. There are, however, distinct cultural characteristics by means of which B. coli can be differentiated from the B. typhosus and B. enteritidis. Among the most important of these are the following, mentioned by Jordan (34):

<table>
<thead>
<tr>
<th>B. coli</th>
<th>B. typhosus</th>
<th>B. enteritidis (Gärtnæ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slightly motile short rod, often difficult to distinguish from micrococci, few flagella. Grows more rapidly in gelatin than B. typhosus. Produces acid and curdles milk. Indol is produced by most strains. Dextrose and lactose are fermented with gas production. Visible growth on potato.</td>
<td>Actively motile rod with numerous flagella. Milk becomes slightly acid but is not curdled. Indol is not formed. Dextrose is fermented but no gas is produced. No acid is obtained from lactose fermentation. Invisible growth on potato.</td>
<td>Actively motile with numerous flagella. Indol is not produced. Milk is not curdled. Dextrose is fermented with gas production but no gas or acid is formed from lactose. Distinctly pathogenic for animals and for man.</td>
</tr>
</tbody>
</table>

The agglutination reaction may also be used to differentiate the members of the colon-typhoid group. As a rule, the blood serum of patients with an acute or chronic typhoid infection will agglutinate and inhibit motility in a hanging drop suspension of B. typhosus, but such a reaction rarely occurs when typhoid serum is used with B. coli, at least in relatively high dilutions. Normal serum may have the power to agglutinate certain
strains of bacilli in low dilutions, such as 1-5 to 1-10. There are certain exceptions to the general rule of specific agglutination, which have been mentioned in the chapter dealing with typhoid infection, but for practical purposes such reactive phenomena to the patients’ sera have considerable diagnostic importance.

Despite the fact that B. coli is a normal inhabitant of the body, no general immunity reactions such as that of agglutination are shown by the blood of normal persons.

Christophers (14) has stated that a large proportion of normal human sera will cause agglutination of B. coli in dilutions varying from 1-20 to 1-200. It would seem if this were universally true that considerable natural immunity was possessed by most individuals toward the organism, which clinically does not seem to be in accord with the facts. In my experience, normal human serum does not possess agglutinins for B. coli. For example, in a recent series of tests, the sera of five individuals did not cause agglutination with loss of motion within one-half hour in dilutions higher than 1-5. On the other hand, the sera of five individuals who had, within one year, received a series of antityphoid vaccinations possessed agglutinins for B. coli within one-half hour in dilution 1-20. The serum of an individual who had typhoid ten years before possessed no agglutinins for B. coli in dilution 1-5, while the serum of one patient with long-standing B. coli bacilluria possessed agglutinins to his homologous strain, following a series of autogenous vaccinations, in dilution 1-20.

On the other hand, when B. coli becomes an inhabitant of the blood stream or of certain organs, there producing symptoms of inflammation with destruction of tissue cells, a reactive phenomenon to its presence occurs with the development of specific agglutinins. Such agglutination is probably specific only for certain groups of strains, since it is well known that not all strains react alike in this respect.

It is not known upon what the varying susceptibility depends. The reaction is probably more or less a group phenomenon, for it is recognized, as mentioned above, that some strains of B. coli will react positively with typhoid blood serum. Such reactions are usually not confusing, for, while sera may give non-specific reactions, such reactions occur in comparatively low dilutions, while in specific reactions to infection, agglutination and paralysis of motility occur in much higher dilutions. For example, in studying the agglutinative powers of a patient’s blood serum toward a strain of organisms isolated from the urine in cystopyelitis, it was found that agglutination occurred in dilution 1-100 in 30 minutes. When the blood serum was tested against a stock strain of B. typhosus, it was found that agglutination did not occur in dilution 1-20 in one hour, while with a stock strain of B. coli agglutination occurred in dilution 1-80 in 20 minutes. If the cultural characteristics were not too much at variance such evidence would favor B. coli as the causative organism in a suspected in-
B. COLI IN TISSUES OUTSIDE OF INTESTINES 235

fection. In my experience, agglutination in dilution above 1-40 has diagnostic significance.

Other organisms, such as B. enteritidis of Gärtner and B. psittacosis, resemble more or less closely the organisms of the colon-typhoid group and may be found in lesions in the tissues. Thus the B. enteritidis may be associated with B. coli, which may seem to be possessed of exalted virulence, in fatal hemorrhagic gastro-enteritis due to eating putrefied meat. It has been contended by some observers that under such conditions the B. coli becomes highly virulent for man in the intestine. The B. enteritidis closely resembles the paratyphoid organism (see below) in that indol is not produced and the fermentation sugar tests correspond.

The paracolon and paratyphoid group, first discovered by Achard and Bensaude (1896), more thoroughly studied by Gwyn (1898), Schottmüller (1901), and Buxton (1902), have been frequently encountered in association with lesions produced by members of the colon group. These organisms of themselves may produce ulcerative lesions of Peyer's patches, although very severe forms of gastro-enteritis without ulceration are occasionally encountered in which these organisms seem to play an etiologic rôle. Two types are recognized, A and B. Type B is probably more widely distributed, and is the organism usually present in so-called paratyphoid fever. The types of B. paratyphosus resemble B. coli in that acid and gas are produced in dextrose media, while they resemble B. typhosus in not causing coagulation in litmus milk (Schorer, 54). The close resemblance of the paratyphosus groups to the bacillus of hog cholera (B. cholerae suis), B. enteritidis, the bacillus of mouse typhoid (B. typhi murium), and B. psittacosis, extends even to similarity in agglutination and immunization experiments.

The B. proteus (Hauser, 1885), which has been occasionally found in association with B. coli in abscesses and in gastro-enteritis, can be differentiated, as a rule, without difficulty. This organism, commonly found in decomposing organic matter, apparently has been responsible for certain endemics of food poisoning. It also has been regarded as the cause of some cases of acute infectious jaundice (Weil's disease). In tuberculous cystitis the secondarily infecting organisms frequently belong to the proteus group, although just why this association occurs, if it is anything more than coincidence, is not known.

TOLERANCE TO B. COLI OF TISSUES OUTSIDE OF THE INTESTINAL TRACT

The constant presence of this organism in the lower intestinal tract in man has been mentioned above. Under certain conditions, which are not difficult to conceive because of the proximity of the urethral opening, espe-
cially in women, the organism becomes an inhabitant of the urinary tract. In fact, during pregnancy or the puerperium, this organism can be isolated from the urine in about 20 per cent. of the cases (Dudgeon, 17). Since its presence does not apparently in the vast majority of puerperal patients produce symptoms or complications, it may be regarded as a normal inhabitant, under certain conditions, of the urinary tract.

That the organism in these instances does not produce symptoms or complications when located in the urinary tract depends upon such factors as (1) virulence of the organism, (2) local cellular resistance or immunity, (3) absence of tissue lacerations or abrasions through which the organism may reach deeper structures.

The first factor, the virulence of the organism, may depend upon the amount of putrefactive disturbance in the intestinal tract giving origin to the infection. Many writers are convinced that B. coli isolated from an intestinal tract in which stasis and putrefaction are present, as evidenced by indicanuria, are more virulent. Symbiosis may enter into the question of virulence and tissue resistance. For example, the association of B. coli and B. typhosus or the toxic products of either accentuates the virulence of the other. Guinea-pigs and rabbits, which may resist the subcutaneous dose of a culture of B. typhosus, quickly die of a generalized infection if a sterile culture of B. coli is injected into the peritoneal cavity. The special susceptibility of tissues may also influence virulence. B. coli isolated from a septic peritonitis are as a rule much more virulent for animals than the strain isolated from the intestinal tract of the same individual.

Of local cellular immunity little is known, although it is recognized that the cells of certain tissues may show greater resistance to certain infections than the cells of other tissues. For example, the pneumococcus is seldom isolated from ordinary furuncles or skin abscesses, nor does it produce lesions of the mucous membrane of the mouth, although it is normally present there in a large proportion of individuals during the winter months. Nor does the B. coli commonly produce furuncles or skin abscesses, even though an abrasion is present, although most individuals come in daily contact in one way or another with the organism.

Of the third factor it may be granted that absence of tissue laceration must prevent, in most instances, spread of the infection to neighboring lymphatics, the blood stream, and distant tissues. On the other hand, when laceration of tissue, even though microscopical in extent, has occurred during pregnancy or the trauma incidental to surgical procedures, the avenue of entrance is established. The experiments of Heinricius (28), which consisted in the injection of bouillon cultures of B. coli into the uteri and vaginæ of rabbits, showed that the intact epithelium prevented infection of the underlying connective tissues. Where the epithelium had been abraded the tissues beneath the epithelium were swarming with organisms. He found that in general there was some simi-
larity between the action of B. coli and streptococci in producing a bacteriemia. Some differences could be seen, however. The B. coli seemed to infiltrate the connective tissues in all directions, disregarding the lymph channels, while the streptococci followed the lymph channels.

THE SPREAD OF THE INFECTION TO OTHER TISSUES

The textbooks of a decade ago referred constantly to an ascending infection of the urinary tract, by which it was intended to imply that infective organisms entered the urethra and by continuity of tissue traveled upward to the bladder and then in many instances to the pelvis of the kidney. It has been practically decided by most authorities that an ascending cystopyelitis is rare. The organisms much more commonly reach the kidney pelvis by way of the lymphatics or blood stream or by continuity of tissue from the colon. Rolleston (48) believes the transperitoneal method of infection from the colon to the kidney by way of the lymphatics to be common. Franke's (21) experiments seemed to show that the ascending colon and cecum were connected by lymphatics with the right kidney, but he was unable to find such a connection between the colon and the left kidney. This work needs confirmation. Rovsing (51) believes in the spread of the infection to the kidneys by the hematogenous route. He had treated, up to 1909, 285 patients with B. coli infections, and in 180 of these the disease arose as an acute nephritis. In no instance had the patients been catheterized.

On the other hand, because of the more or less constant presence of bacteria including B. coli in the vagina, it is not difficult to understand the manner by which they reach the bladder through the short urethra of women and children. Their presence there does not necessarily mean an inflammatory reaction and cystitis. In my experience, however, B. coli has been found more frequently in cystitis than any other organism. It has been found frequently in association with the tubercle bacillus in tuberculosis of the kidney and bladder. Likewise it is the organism frequently found in the urine when calculi are present in the pelvis of the kidney, ureter, or bladder. Ohlmacher (42) has recently reported the presence of B. coli in five out of eight instances of bacteriuria associated with urinary calculi. The more or less frequent presence of B. coli in leukorrheal secretions would seem in some instances to be a possible cause of sterility, since an excessively acid secretion would inhibit the activity of, if it did not kill, spermatozoa. This fact has been emphasized by Morris (40). Considering the apparent ease with which an infection by B. coli can reach the uterine cavity and tubes from the vagina, it is somewhat surprising that this organism is relatively so infrequently encountered as the causative factor in ovarian abscess and tubal infections. Grover (24) has re-
cently described fatal peritonitis due to B. coli which followed perforation of the uterus in a probable attempt to produce abortion. Peritonitis following perforative appendicitis and subphrenic or liver abscesses following cholecystitis are frequently due to B. coli.

An attack of pelvic peritonitis may follow infection with B. coli from the vagina by ascent through the uterus without, so far as the patient is concerned, provocative cause. In fact, lacerations of the hymen serve as the entering point of infection to the bladder, kidney pelvis, and peritoneum through the lymphatics in more instances than are generally recorded. Such a sequence in a recently married woman is commonly ascribed to the organism associated with specific urethritis. Wildbolz (67) has recently described infection of the bladder and kidney pelvis in eight such cases by B. coli. Murray, Williams, and Wallace (41) found B. coli present in 44.5 per cent. of gynecologic cases with normal urine prior to operation and in 93 per cent. subsequent to operation. There appeared to be no relation between post-operative temperatures and the presence of the bacillus in the urine.

SYMPTOMS OF INVASION OF THE GENITO-URINARY TRACT
BY B. COLI

Urethritis.—Normally in healthy women or men the urethra is uncontaminated, but if any damage has previously been done to the lining membrane by trauma incident to child-bearing, by the forceful passage of a sound, by infection from an unclean catheter, by gonococcus infection, or as the result of irritation due to the constant passage of infection from above, such as tubercle bacilli from an infected kidney or bladder, then and subsequently contaminating organisms are frequently present in the urethra. The two most common organisms found in non-specific urethritis are the staphylococcus albus urethrae and B. coli. In fact, these organisms may be present in the urine with no urethral symptoms. Dudgeon has stated that he has never seen an acute B. coli urethritis in men. Sherck's (52) experience has been similar. Koll (36), on the other hand, found B. coli as a secondary infection in six out of twelve cases of urethritis. Chronic gonorrheal urethritis is often prolonged by the presence of B. coli. Reynolds (49) has emphasized the importance of the B. coli in the production of epididymitis secondary to chronic gonorrheal posterior urethritis.

Cystitis.—In simple primary cystitis due to B. coli the symptoms are usually not severe, but point, as a rule, to local involvement of the posterior urethra and bladder. In males the prostate gland and seminal vesicles may be involved. The symptoms are frequent desire to empty the bladder and a burning or scalding sensation along the urethra, associated with the passage of small quantities of acid, turbid urine. Examination discloses,
as a rule, some tenderness in the rectum, and in males the prostate gland may be swollen and tender. In women leukorrhea may be present. The urine contains many polymorphonuclear leukocytes, as a rule, although pus may be absent and the turbidity be due to the large numbers of bacilli suspended in it. The acidity is usually much increased, varying from 600 to 800 per 1000 in terms of decinormal sodium hydride. Cultures taken from urine secured under aseptic precautions upon nutrient agar disclose a rapidly growing, slowly motile rod, which conforms to the cultural characteristics mentioned above. The most important of these characteristics are acid production and curdling of milk, the production of indol, gas production in gelatin stab, and fermentation of dextrose and lactose with acid production. The organisms grow and reproduce rapidly at room temperature, although at 37° C. their reproduction is most rapid. Barber (4) found the generation rate to be seventeen minutes at 37° C.

If the urine to be examined culturally is secured from a female, the specimen should be secured in a sterile glass through a sterile catheter and after thorough cleansing of the urethral orifice. As ordinarily performed, it has always been a wonder to the writer why more infections of the bladder do not occur following catheterization, since the B. coli so commonly contaminates the pubic region and the urethral orifice. Such bacteria on the surface are introduced regardless of the care used. In males quite so much precaution in securing the specimen need not be observed, although the urethral orifice should be cleansed with a mercury bichlorid or phenol solution. The first portion of urine passed should be discarded and the remaining portion collected in a sterile glass, from which the culture should be taken. Acute cystitis in children has, in my experience, frequently been due to B. coli. As a rule, fever is not present in simple uncomplicated cystitis. If fever is present, it speaks for involvement or extension of the infection elsewhere, most commonly pyelitis, to the pelvic peritoneum or ovaries, the prostate gland or seminal vesicles. Ulcerative lesions in the bladder due to B. coli are rarely seen in acute cystitis, but may occur in chronic forms associated with stone and sacculcation.

Pyelitis.—According to Billings (5), the B. coli is found as the infective organism in about 50 per cent. of all cases of bacteriuria. Lenhartz (38), however, found B. coli alone in 66 out of 80 patients with pyelitis (75 per cent.). The infection may reach the kidney pelvis by ascension from urethra and bladder (urogenic), by the blood stream (hematogenic), from the intestine (transperitoneal), or by way of lymphatics from some focus of infection in the neighboring tissues. A vast majority of instances occur in women; thus Lenhartz (loc. cit.) found 74 instances in women out of 80 primary pyelitis cases; most instances occurred after pregnancy or childbirth. Malformation or displacement of the kidneys seems to predispose to infection. The right kidney is much more frequently affected than the left. In 65 out of 70 cases (93 per cent.) the right kidney was in-
volved, according to the experience of Legueu (37). The acute onset is usually severe, with rigor and fever from 101° to 104° F. Tenderness may be present by palpation over both kidneys at the costovertebral angles (Brewer’s point), although the tenderness is usually more marked over one side. The spleen is usually palpable. A leukocytosis of from 12,000 to 25,000 is usually present. There is constant desire to void urine, which is turbid from pus and bacteria and acid in reaction. The urine is, as a rule, voided painlessly.

A majority of the instances of kidney involvement follow operative procedures. Fenwick (19) has described the frequency of pyelitis following operations for hemorrhoids, which he has ascribed to the following causes: (1) surface lesions in an infected area; (2) congestion of the vesical neck; (3) retention of urine.

The infection may reach the kidney pelvis through extension along the periureteral lymphatics. Sugimura (62) has described the conditions found in twenty-five patients with cystitis. He believes that, although the urethral orifices may be reddened and apparently involved, extension upward of the infection occurred in all by way of the lymphatics and not by ascension through the ureters. The fact that many instances of post-operative kidney infection occur in which there has never been an antecedent cystitis or catheterization lends support to the view of extension by way of the lymphatics or blood vessels.

Furniss (22) believes the late occurrence after operation lends weight to the belief that the origin of the infection is from thrombi at the site of operation. While the symptoms are usually suggestive of kidney involvement, such an infection may be mistaken for other acute conditions, such as appendicitis, peritonitis, cholecystitis, pelvic vein thrombosis, prostatic abscess, seminal vesiculitis, or an ether nephritis. A true infectious nephritis usually accompanies the pyelitis. In fact, in most instances, the infection by way of the blood stream reaches the kidney pelvis through the kidney parenchyma. The only exceptions would appear to be those in which the infection reaches the kidney pelvis by extension upward along the periureteral lymphatics or by ascension through the ureter in rare cases. Localization of the infection in the parenchyma with miliary abscess formation may accompany the pyelitis. As has been pointed out by Rovsing (loc. cit.), the small miliary abscesses closely resemble macroscopically, miliary tubercles present in tuberculosis.

The acute stage of pyelitis, with continuous or remittent high fever, perhaps with recurring chills, although more commonly a single initial chill occurs, lasts usually from one to three weeks. Hematuria occurs more or less frequently as an initial symptom, and is soon followed by albuminuria, pyuria, and bacilluria. Casts are not usually found in pyelitis, a point emphasized by Abt (1) and Wassermann (65), but are present in pyelonephritis. The fever disappears, in some instances by crisis, al-
though in probably three-fourths of the cases the fever gradually becomes remittent and disappears by lysis. With the disappearance of the fever the urinary findings may improve; that is, the amount of pus may diminish and there may be a decrease in the number of bacilli, but, as a rule, there is no essential change in the bacilluria. After an afebrile period of from 3 to 10 days a relapse may occur or a new attack involving the hitherto intact kidney pelvis may take place. In fact, febrile disturbances may alternate with afebrile intervals over a considerable time until the process becomes chronic, or the pyuria and bacteriuria may become chronic without marked local symptoms, such as dysuria or pain. If obstruction to the free flow of urine has occurred, as a result of calculi, prostatic swelling, sacculcation or atony of the bladder, ureteral kink, or pressure obstruction, symptoms such as fever and rigors promptly follow. It is surprising, however, what large quantities of pus may be eliminated in chronic infections involving the kidney pelvis over periods of months without great apparent harm to the kidney structure. In many instances the pyuria and bacteriuria continue for months, and aside from some loss of weight, strength, and appetite, with the development of pallor due to secondary anemia, there are few subjective or objective symptoms.

Thomson (63) believes that the commonest occasion of acute septic invasion of the kidneys by B. coli is in the course of typhoid fever. Chronic ulcerative colitis is another common antecedent. The onset during typhoid is sometimes marked by severe rigors. Their occurrence during the course of typhoid fever should raise the suspicion of acute septic invasion of the kidneys by B. coli. The temperature usually becomes irregular and the quantity of urine diminished. Coleman and Hastings (15) have emphasized the fact that some strains of B. coli are capable of producing a generalized infection clinically identical with typhoid fever. The occurrence of an acute infectious nephritis due to B. coli becomes immediately a serious condition if the patient has previously had evidences of chronic interstitial changes involving the kidneys. In such conditions the invasion may be preceded by an attack of colitis, acute appendicitis, or gastro-enteritis. The urine then becomes decreased in quantity with a tendency to suppression. The presence of fever and an initial rigor frequently lead to a suspicion of pneumonia. Delirium is usually rapidly followed by coma and hyperpyrexia. An acute infectious nephritis due to B. coli terminates a chronic interstitial or diffuse nephritis more commonly than text books and recent literature would lead one to suppose. I have seen a number of such instances of sudden onset with enormous numbers of B. coli in the urine.

Acute infectious pyelonephritis due to B. coli may occur during the course of measles, diphtheria, and scarlatina. In such instances, especially in children, the symptoms such as vomiting and abdominal distention may present the picture of an "acute surgical abdomen," and appendicitis, in-
testinal obstruction, volvulus, or mesenteric thrombus may be suspected. The symptoms may, on the other hand, present the picture of a generalized infection with little pain or tenderness in the kidney region, and as such simulate influenza, typhoid fever, or septic endocarditis. In children and chlorotic girls, because of recurring mild febrile attacks, tuberculosis or chronic tonsillar infection is often suspected.

In pyelitis occurring during pregnancy the B. coli is frequently found to be the causative organism. It is surprising how serious the condition of the patient may appear to be and recovery take place. If the only symptoms are moderate fever, pyuria, and albuminuria without suppression, the patient will usually weather the storm until confinement, even though enormous quantities of pus are present in the urine. When, however, partial suppression occurs, due to the infectious nephritis and stagnation of urine in the kidney pelves, with chills and high fever, the question of artificial delivery to relieve the retention becomes paramount. If the patient has but a few weeks to full term and the infectious process has not existed long enough to produce the appearance of sepsis, with secondary anemia, vomiting, and general malnutrition, it may be wise to wait in order to obtain added security for the fetus. When partial or complete suppression occurs in long-standing infections of this character, if not quickly relieved, it becomes necessary to resort to artificial delivery in order to relieve the retention and save the patient, even though the child be sacrificed. In a few instances, in my experience, vaccine therapy has been of decided value, but these were of the type without suppression (see below). The vaccines seemed to control the fever, without which the condition of the patient's nutrition improved to such an extent that it was possible to await full-term delivery, even though enormous quantities of pus were present in the urine.

**DIFFERENTIAL DIAGNOSIS**

The diagnosis of uncomplicated chronic B. coli pyelitis or pyelonephritis will depend upon the isolation of B. coli from the urine and the exclusion of other organisms which may produce similar symptoms, such as the staphylococcus, streptococcus, or B. proteus.

The staphylococcus may produce pyelitis or pyelonephritis identical, so far as the clinical picture is concerned, with infection by B. coli. The chronic form of such infections, or infection with B. proteus, produces symptoms of severe intoxication. The more or less constant fever, sallow appearance, history of considerable loss of weight, headaches with loss of strength and appetite often cause a suspicion of typhoid fever. [The possibility of typhoid fever with an onset resembling acute nephritis must be considered in such cases.—Editors.] The presence of pyuria, with more
or less albuminuria in the filtered specimen, the ready cultivation of staphylococci or B. proteus upon agar, together with a leukocytosis from 12,000 to 20,000 per c. mm. and negative typhoid agglutination reactions. usually promptly clear up the diagnosis. The presence of stone is frequently suspected, and with justification, even though no attacks of colic or hematuria have occurred. Roentgen-ray examinations are an important aid.

Staphylococcus and streptococcus infections of the kidney structure more frequently follow an angina or scarlatina, or some acute infective process, such as glandular suppuration, peritonsillar abscess, carbuncles, or osteomyelitis than infection with B. coli. B. proteus, as has been mentioned above, is more frequently found in association with the tubercle bacillus in tuberculous nephritis. Staphylococci and streptococci in urine have the power to decompose urea. Such specimens have a strong ammoniacal odor and are alkaline in reaction. Since the bacteria are non-motile, they settle to the bottom of the container along with pus cells, crystals, and epithelial cells, leaving the supernatant urine clear. The B. proteus likewise has the power to decompose urea. Such urine has a strong ammoniacal odor, and is alkaline in reaction, but since the B. proteus is actively motile the organisms do not settle to the bottom of the container upon standing, and the specimen remains turbid. B. coli infection produces an acid urine which remains turbid upon standing because of the active motility of the organism. Infection by B. proteus can usually be differentiated from infection by B. coli by finding, as has been pointed out by Rovsing (loc. cit.), abundant crystals of triple phosphate due to the presence of urea decomposition. The presence of the tubercle bacillus can usually be determined by drying the sediment secured from centrifugated urine upon a glass slide and employing appropriate stains. The bacilli when present occur in small groups. The possibility of confusion with smegma bacilli should be borne in mind, but these can be differentiated by submerging the slide in weakly acidulated alcohol, which decolorizes the smegma bacillus.

Other Lesions Produced by B. Coli.—Chronic gonorrheal urethritis may be prolonged by the B. coli as a secondary infection, which reaches the urethra through unclean instrumentation by the physician or unclean urethral syringes so frequently used by the patient. The infection may reach the posterior urethra and prostate, and by extension through the ejaculatory ducts involve the epididymis. Reynolds (loc. cit.) has recently emphasized the occurrence of epididymitis due to B. coli. Von Schrötter and Weinberger (56) have observed B. coli in the sputum of a patient with a long-standing bronchopneumonia. Pearson (44) has reported B. coli in the cerebrospinal fluid of a fatal case of meningitis, which apparently followed suppurative otitis media. Hartwich (27) also found B. coli in the cerebrospinal fluid of a patient with tuberculous ulceration of the in-
testine. W. S. Stone (60) believes B. coli to be responsible for a few fatal generalized infections during the puerperium. I have never personally seen such an instance.

TREATMENT OF CYSTITIS AND PYELITIS DUE TO B. COLI

Cystitis.—The use of an autogenous B. coli vaccine in cystitis due to this organism, without infection higher up in the tract, has been followed by good results in my hands in numerous instances. The condition seems to be most common in women with relaxed vesicovaginal walls following the trauma of childbirth. Salol, gr. 30 to 45 (2.0-3.0 gm.), daily in adults with large quantities of distilled water (3 quarts daily) has been used as an aid to the vaccine treatment. Hexamethylenamin gr. 10 (0.65 gm.) with acid sodium phosphate gr. 30 (2.0 gm.) three or four times daily, while apparently efficient in bladder irritation due to B. typhosus, has not been followed by such satisfactory results in the treatment of B. coli cystitis. Hexamethylenamin may cause renal and vesical irritation if used for long periods. It may also cause reduction of Fehling's solution, simulating a sugar reaction. This has also been Rolleston's experience. The dosage of vaccine which has seemed most efficient has been 50- to 100,000,-000 at 4- to 5-day intervals.

Pyelitis.—The acute symptoms of uncomplicated pyelitis usually promptly subside when rational treatment is instituted. This should consist of rest in bed, a liquid diet, large quantities of water, distilled preferred, and salol or hexamethylenamin internally, 45 to 60 gr. (3.0-4.0 gm.) daily. The so-called alkaline treatment of citrate of potash or soda bicarbonate is preferred by some clinicians. The bacilluria may, however, persist, and because of recurring acute exacerbations the condition becomes chronic, despite the treatment. In such instances the patients present a sallow cachectic appearance, periodic fever exacerbations occur, hematuria may recur, and malignancy, tuberculous nephritis, and stone are frequently suspected.

If the pyelitis is complicated by conditions which interfere with free drainage and thus favor retention, such as ureteral kinks or stone, prostatic hypertrophy, stricture, atony of the bladder, or pressure arising from uterine or ovarian neoplasm, little may be expected of any treatment except amelioration of symptoms until such conditions are corrected.

Vaccine therapy should be tried in all cases of uncomplicated pyelitis. Cabot (12) found that improvement in clinical symptoms occurred in about 50 per cent. of the cases of chronic B. coli bacilluria treated by vaccines. Geraghty (23), on the other hand, found no improvement which could be attributed to vaccine therapy in the treatment of urinary tract infections. Scherck (loc. cit.) has also found vaccine therapy with both
stock and autogenous strains of little service. On the other hand, the results occasionally obtained warrant the trial of vaccine therapy in any condition not amenable to other measures. Such an instance among others within my recent experience may be cited. (59.)

At about the fifth month of pregnancy this patient began to pass large quantities of pus and blood with the urine. The daily temperature ranged from 101° to 103° F., with occasional chills. This condition was not amenable to any form of treatment during one month by the attending physician. At this time cultures taken from the urine showed B. coli in pure culture. The patient presented a sallow cachectic appearance, while the vomiting and malnutrition incident to the febrile disturbance made the outlook unfavorable to the completion of term. An autogenous vaccine was prepared from the cultures. After the second dose of 50,000,000, the temperature dropped abruptly by crisis to normal and there remained. In all ten or twelve injections were given, and, although the pyuria and bacilluria did not disappear until after the retention was relieved at term, her general condition improved with the disappearance of the fever, and she was delivered of a healthy child. Her complete recovery followed.

Hicks (30) has reported the successful use of B. coli vaccine in the treatment of a patient with pyelitis of pregnancy. As in the case cited above, the fever, which had been more or less constant, dropped almost immediately after the first inoculation, and remained normal thereafter. The pain also rapidly subsided, and the patient's general condition improved. The pyuria persisted for some time and was intermittent in character. Billings (loc. cit.) believes vaccine therapy of decided value in the treatment of B. coli infection of the urinary tract.

[In two cases of pyelitis, occurring in the third and fourth month of pregnancy respectively, spontaneous abortion followed the inoculation of moderate doses of colon vaccines. The relation of the abortion to the inoculations may have been coincidental.—Editors.]

Some strains of B. coli, if used for the preparation of vaccines, cause relatively severe local and general reactions. It is therefore wiser, until the toxicity of the strain has been determined by clinical trial, to start with relatively small doses of 25- to 30,000,000. Some of the strains produce less reaction in doses from 100- to 200,000,000 than others in doses of 25,000,000.

It is possible to render the vaccine less toxic by suspending the bacterial cells, after standardization, in salt solution for 40 to 48 hours at 37° C. in the incubator. Autolysis takes place and the salt solution becomes toxic. The cells are thrown down by centrifugation, the toxic salt solution discarded, and the bacterial cells resuspended in fresh salt solution containing 0.35 per cent. phenol. In my experience the best results have been ob-
tained by gradually increasing the dosage, depending upon signs of local and general reaction, from 25- to 200,000,000 at 4- to 5-day intervals.

In chronic uncomplicated pyelitis due to B. coli, if satisfactory results are not obtained through the combination of salol with the copious ingestion of water and the use of an autogenous vaccine, it may be necessary to resort to the method of continuous drainage, the "catheter à démeure," as advocated by Røvsing (loc. cit.) in 1897. This consists in putting the patient to bed and securing continuous drainage by means of a Mercier's formalin-sterilized rubber catheter in the bladder, in addition to the treatment outlined above. The formalin sterilization hardens the rubber, and it may remain in the bladder for from 3 to 4 weeks without change. When cultures show that the catheter may be removed Røvsing recommends, immediately prior to removal, the injection of 50 c. c. of 1 per cent. solution of silver nitrate in order to rid the bladder of any bacilli concealed in the vesical folds. For women Røvsing recommends a Pezzer's catheter, No. 22 or 23, which is easily kept in place.

This treatment is founded upon the fact that continuous drainage is necessary in chronic pyelitis or pyelonephritis to rid the tissues of bacilli through the urine, since the time which may elapse between urinations is sufficient for the development of enormous numbers of bacilli. Reinfection of the upper urinary tract may thus constantly occur through the lymphatics from the connective tissue of the bladder. As has been mentioned above, Barber found the generation time of the B. coli to be seventeen minutes at body temperature. The only difficulty encountered is to secure enough time to complete the cure, since many patients object to a period of three to four weeks in bed.

TREATMENT OF INFECTED WOUNDS BY B. COLI VACCINES

In about twenty-five infections of the drainage tract following appendicitis and cholecystitis due to B. coli, treated by autogenous vaccines, the results have been satisfactory. The patients were discharged in shorter time than was possible in patients not so treated, due to the lessened fever and wound discharge following the bacterial inoculations. There were also fewer complications during the course of vaccine treatment. In a few patients the discharge ceased after two or three inoculations.

Hoobler (31) has reported the use of B. coli vaccine in a patient following evacuation of a pelvic abscess. The dosage was gradually increased, twice weekly, from 25- to 200,000,000. In all thirteen inoculations were given. The patient made a slow but complete recovery. Stoner (61), in his résumé of vaccine therapy, mentions three patients operated for cholecystitis with gall-stones, in which B. coli infection complicated the recovery. The vaccine inoculations seemed of value in all.
REFERENCES

Autogenous vaccines have given me more satisfaction than stock vaccines, since there was no question as to the particular strain against which it was desired to produce an immunizing response. In the preparation of the vaccine the organisms are grown for 18 to 24 hours upon agar slants. The method otherwise follows the procedure given for the preparation of typhoid vaccine (Chapter VI). Vaccines sensitized by the addition of an immune serum and subsequently killed produce less constitutional reaction when injected and should be preferred for intravenous administration if such a method is selected. The immunity produced has not, however, in my experience, been greater than that produced by the ordinary nonsensitized vaccine. Much more rapid absorption of the vaccine occurs after an intramuscular injection than occurs after a subcutaneous injection and should usually be the method of choice. The injection of living sensitized vaccines at the present time does not appear warranted.

REFERENCES

17. Dudgeon, L. S. Lancet, 1908, i, 615.
42. Ohlmacher, A. P. J. A. M. A., 1913, lx, 1213.
52. Scherck, H. J. Personal communication.
54. Schorer, E. H. Vaccine and Serum Therapy, 1913, p. 207.
CHAPTER VIII

BACILLARY DYSENTERY

RICHARD P. STRONG

THE BACILLUS OF DYSENTERY

Types of Dysentery Bacilli.—In order to have a proper understanding of the subject of the vaccine and serum treatment of bacillary dysentery, it is necessary to consider the etiology of the disease, and the immunizing properties of the organism and the action of the serum produced with it. By the term, “bacillary dysentery,” we understand an infectious form of dysentery caused by one or more members of a group of closely related micro-organisms, of which bacillus dysenteriae (Shiga) may be regarded as the type. This organism was first definitely shown to be the etiological factor in this disease, and the several other forms of bacilli, discovered shortly afterward, and also sometimes shown to be the causative agent, may be considered as different varieties or types of this organism. Considerable difference of opinion, however, has been expressed in regard to the classification and separation of the original types or varieties of dysentery bacilli.

In December, 1898, Shiga first described bacillus dysenteriae as the cause of a form of dysentery in Japan, and in March, 1900, Flexner isolated the type which has since come to bear his name. In May, 1900, the author, during the study of a severe epidemic of dysentery in Manila, isolated, along with other strains of the dysentery bacilli, one which showed certain variations from the others, and which also has gradually come to be designated by his name. In 1900 Kruse, in Germany, also described an organism as the cause of dysentery identical with the Shiga type. Three years later (1903) Hiss and Russell, in a study of the types of dysentery bacilli mentioned above, described another one, which they encountered in a case of intestinal disease, and which has since been shown to be rather closely related to the Flexner type of organism. To this organism the term bacillus Y has been applied. Flexner and Shiga at first believed that the organism isolated by Flexner from dysentery cases was identical with bacillus dysenteriae Shiga.

Kruse first pointed out the possibility of the differentiation of the dif-
ferent types of dysentery bacilli by means of the agglutination test, and
classified these organisms into "true" and "pseudo" dysentery bacilli. He
showed that Flexner's bacillus was not identical with the Shiga-Kruse
bacillus, but agreed closely in its serum reactions with the organism
which he named "pseudodysentery bacillus of insane asylums." The term
"pseudodysentery bacillus" has not been maintained, as this type of or-
ganism also has been shown to cause dysentery in man.

Martini and Lentz, in addition, showed that the Flexner and Strong
strains could be differentiated from the Shiga ones by cultural tests on
media containing mannite, as well as by specific agglutinating sera, and
also that the Flexner and Strong strains were not identical. Hiss and
Russell demonstrated that the strains, Shiga, Flexner, Strong, and bacillus
Y, all presented distinct cultural differences from one another.

Flexner and his assistants, by whom most of the work upon this sub-
ject in the United States was performed, have recognized two main types
of dysentery bacilli, the Shiga and the Flexner ones, and also several sub-
sidiary types. Gay, working in Flexner's laboratory, found that the dysen-
tery bacilli fell into two types, according to certain cultural, bacteriolytic,
and agglutinating reactions.

Shiga, however, separated the dysentery bacilli by cultural reactions
and serum tests into five types. The first four types accorded entirely
with those of Hiss. The fifth type differed from the fourth in that, after
24 hours, it gave an acid reaction in a culture medium containing man-
nite; but this gradually disappeared, until finally, after 4 days, the me-
dium became alkaline, and remained so. He regarded this fifth type as
an intermediate one between the acid (fermenting) and non-acid dysen-
tery bacilli.

Among the different strains of dysentery bacilli isolated by the writer
in Manila, as has been confirmed by Shiga and later by Lentz, both acid-
and non-acid-forming strains were present. Shiga points out that it is
probable that the discovery of a non-acid-forming variety of bacillus as the
cause of dysentery in Japan was accidental, and also that it seems purely
accidental that the acid-forming variety was first discovered as the cause
of dysentery in Manila. In epidemics in Japan both the acid and non-
acid varieties were encountered.

Park and his assistants called attention to the fact that all varieties of
dysentery bacilli which produce indol in large amounts, and which develop
acid from mannite, distinctly differ in their agglutinative reactions from
those which do not act upon mannite, and which produce no indol, or only
a trace of that substance. By agglutination and absorption tests they sepa-
rated the dysentery bacilli into at least three types or varieties.

On the other hand, Ohno, working upon a large number of freshly iso-
lated strains, many of which were obtained from severe cases of the epi-
demic disease, believed that 15 varieties could be distinguished. He
studied 74 strains of dysentery bacilli. He did not consider that there was any advantage in separating the dysentery bacilli into two distinct groups—an acid and non-acid—as proposed by Lentz, as he found that the grouping of the different organisms according to the differences in their powers of causing fermentation does not correspond to that which results from differences observed in agglutinative and bacteriolytic action with specific immune sera. The antidysenteric rabbit sera, prepared with so-called non-fermenting bacilli, often agglutinate strains which ferment mannite in the same or in higher dilutions than they do other organisms of the non-fermenting type, and vice versa. The same phenomena could also be confirmed by bacteriolytic tests, the so-called Cross bacteriolysis usually taking place between bacilli termed both acid and non-acid.

Ohna's conclusions, however, are exceptional, and from the more important recent investigations carried on in various parts of the world the four types of the dysentery bacillus appear now to have become definitely established. The recent publications of Morgan (1911), Martini, M. Wassermann, Butler (1912), Lentz (1913), and Rodenwaldt (1914), emphasize further the differentiation of the dysentery bacilli into the four types as originally proposed by Hiss and Russell.

Morgan, in 1911, clearly demonstrated the division of the dysentery bacilli into these four groups. He showed, however, that the Y bacillus of Hiss and Russell was far more closely related on biological and serological grounds to the Flexner bacillus than was the bacillus Strong. He also pointed out that, in the mannite-fermenting dysentery group, excluding the Strong strains, must be incorporated a large and probably ever-increasing number of strains reacting with striking uniformity to one test—that is, agglutination with Y or Flexner serum—but differing from one another markedly when their fermentation properties and the receptor mechanisms are minutely investigated.

It has also been shown subsequently that a serum prepared with the Flexner organism often agglutinates bacillus Y in as high dilution as it does the Flexner one, and, while bacillus Y may be regarded as a subsidiary type of the Flexner organism, the bacillus Strong type evidently differs more widely.

Duval and Bassett reported that the so-called summer diarrheas of children which prevail in temperate climates are frequently caused by the dysentery bacillus. Charlton and Jehle cultivated dysentery bacilli of the Shiga and Flexner types from children suffering either from dysentery or with what they called a form of food poisoning, but failed to find the organism in a group of children suffering with summer diarrhea. They also obtained a Flexner type from two healthy children, an observation which has been confirmed by Duval and Schorer.

For a short time after the four different types of dysentery bacilli had been described, some doubt was expressed as to whether all of them were
really capable of producing dysentery in man. The author was able, in 1900, first to demonstrate conclusively that this fact was true in regard to the organism isolated from his own dysenteric cases, and subsequent investigations, in which infection occurred among laboratory workers, have demonstrated that the same is true for the other three types.

Differentiation of the Four Types of Dysentery Bacilli.—It is not within the scope of this paper to discuss all the evidence regarding the differentiation of the dysentery bacilli into the four types or groups. This subject very recently has been considered extensively by Lentz (1913). From a cultural standpoint the four types may be separated definitely by their growth upon the various media containing sugars. The following table, from Kolle and Wassermann's "Handbuch," shows at a glance the more important distinctions:

<table>
<thead>
<tr>
<th>Litmus Agar with Addition of</th>
<th>Appearance of the Culture Inoculated with Bacillus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shiga-Kruse</td>
</tr>
<tr>
<td>Mannite</td>
<td>Blue</td>
</tr>
<tr>
<td>Maltose</td>
<td>Blue</td>
</tr>
<tr>
<td>Saccharose</td>
<td>Blue</td>
</tr>
</tbody>
</table>

This classification has been generally accepted. Hiss and Shiga formerly found that the Flexner type also gave rise to acid production in saccharose, but this has not been confirmed subsequently by Lentz, Lieffmann, Nieter, Butler, and others. It has been shown, however, by Hiss, Lentz, and others that the type Y may after long growth on artificial media sometimes ferment maltose, as does the Flexner type.

The different types may also be separated by the agglutination test, a specific serum prepared with each type being used for the purpose. As already pointed out, however, the Y type sometimes shows agglutinating reactions almost identical with the Flexner type. Gay found for the two types studied that perfect bacteriolysis depended upon the employment of bacilli and immune sera of corresponding type, and hence this method may be used to distinguish them. The absorption method of Castellani has also been employed for the purpose of assisting in separating the types. It has been stated that the absorption of agglutinins from an immune dysenteric serum by any of the bacillary types will remove the major or minor portion of the agglutinins present, depending upon the type of bacillus used in immunization. The so-called major agglutinins are the more specific ones, the minor agglutinins being produced indefinitely by any of the types, or by similar ones. The so-called "common" agglutinins have been shown to be present in normal blood, for example, of the horse.

Wassermann, however, has recently shown that the absorption method
and other serological procedures, such as the complement-fixation and opsonic reactions (formerly recommended by Händel for the differentiation of the types) as well as the anaphylactic tests, are much less reliable than the simple agglutination test taken in connection with the cultural tests. The absorption method he found particularly unreliable. These observations, in the main, have been confirmed by other observers.

**Classification of Types by Toxicity.**—In the United States, however, there has been a tendency among a number of authors to disregard the separation of the dysentery bacilli into the four main types, and to classify them merely as the acid and non-acid varieties. This, while a much simpler and easier classification for the investigator, has naturally led to some confusion, particularly where the types encountered have not also been carefully differentiated by serum reactions. There is still considerable difference of opinion as to the extent or variation which each type may exhibit. In some cultures of the Y bacillus, as has been mentioned, a very close relationship with the Flexner type has been observed. Dopfer's cultures showed considerable variation, and Muller believes he has shown true mutation in the dysentery bacilli. Hutt, although he states that the different types are not really maintainable, if the sugar reactions are taken as a criterion, was unable to obtain a transition from the type bacillus dysenteriae to the type which he calls pseudodysenteriae.

Some observers have attempted to classify the dysentery bacilli into a toxic and non-toxic group, placing the Shiga-Kruse organism in the toxic group, and the Flexner, Y, and Strong bacilli in the non-toxic one. This classification, however, is, in some respects, erroneous. Kraus and Doerr pointed out that not all strains of the Shiga-Kruse type give rise to a satisfactory toxin for the purpose of serum production, and they found that the strains which produced a rather characteristic pellicle in fluid culture media were particularly toxic. Moreover, the type Strong was, at the time of its isolation, extremely toxic, and was used in 1900-1901 to prepare a serum for therapeutic purposes, which was employed with good results in the treatment of severe cases of dysentery.

**Differentiation of Types in Relation to Treatment.**—The classification of the dysentery organisms, and their differentiation into types, obviously have been considered in this article from the standpoint of treatment with specific sera. Gay showed that each immune serum is most active against its own type of organism. If the organism giving rise to the dysentery produces a potent toxin then, if an antitoxic serum is prepared with this same organism and employed in treatment, favorable therapeutic results may be expected. It should also be borne in mind that, if the serum is prepared with the same type of organism causing the disease, its bactericidal effect upon that organism will be much more marked than if an organism of another type is used in its production. Theoretically the most favorable results in serum treatment should be obtained
when an individual is treated with the type of serum prepared with an organism of the same type as the one producing the infection. Recent work, particularly that in Egypt by Ruffer and Willmore, has confirmed this idea by the practical results obtained in treatment with serum. For this reason, in countries where the disease occurs frequently, it would appear advisable to prepare sera for treatment corresponding to the four main types of the organism. Flexner and his assistants, Kruse, Dopter, Ohna, Amako, Ruffer, and others, have shown that more than one type of organism may occur in the same individual. In such cases polyvalent sera should preferably be employed for treatment. The most satisfactory results could not be expected from a univalent serum. Unless the type of infecting organism in any given case is known, polyvalent sera should always be employed.

THE DYSENTERY TOXIN

Shiga first showed that the injection of the dysentery bacillus into rabbits produced death, and that toxic lesions were present in the large intestine.

The dysentery toxin was first obtained by Conradi. He allowed cultures of the organism to undergo autolysis at 37° C., filtered through a Berkefeld filter, and concentrated the filtrate by evaporation. One-tenth c. c. of such a preparation was fatal for rabbits in 48 hours, the injections causing, prior to death, diarrhea, collapse, and paralysis. In 4 animals, which lived longer than 48 hours, diphtheritic inflammation of the intestine and ulceration were observed.

Neisser and Shiga, in a somewhat similar manner, also demonstrated the action of the toxin of the dysentery bacillus. Cultures of the organism were suspended in saline solution, killed by heating, the suspension kept for 2 days at 37° C., and then filtered through a Reichel filter. Neisser and Shiga noted the same symptoms in animals after the injection of this filtrate as did Conradi, with the exception that the lesions of the alimentary tract were largely confined to the small intestine.

Vaillard and Dopter found that, when the rabbits died after the injection of small doses of the toxin, in from 18 to 24 hours lesions of the small intestine were observed; but, when larger doses were injected subcutaneously, lesions of the large intestine appeared.

Todd and Rosenthal investigated the toxins of the dysentery bacillus by growing the organism in bouillon for several weeks. Todd found that the filtrates from ordinary broth cultures of the bacillus are only slightly toxic for animals, but that, by using a more alkaline medium, much more highly toxic filtrates could be obtained. The best results as regards the toxicity of the filtrates appeared to be reached in about a month to six
weeks. After this the toxicity began to fall. The toxin appears to be moderately heat-stable. Neisser and Shiga found that a temperature of 75° C. reduced its action greatly. Vaillard and Dopter found that one of 75° to 80° C. was necessary to diminish the toxicity, while at 81° C. it was destroyed. Todd states that the toxin is not destroyed by heating at 70° C. for 1 hour, though exposure to 80° C. for 1 hour seems to destroy it entirely. Rosenthal found the toxin to be weakened by heating at 70° C., while Flexner found it was injured at 80° C. and destroyed at 81° C. for 1 hour.

Kraus and Doerr studied the action of filtrates of 10-day-old bouillon cultures and of saline extracts of fresh cultures of the dysentery bacillus. In their opinion no autolysis is required, since the living bacilli yield the toxin by extraction with salt solution.

Ludke ground the bacilli at a temperature of liquid air, according to the method of Rowland and MacFadyan, followed by extraction with salt solution. In this way a toxin was obtained which caused, chiefly, lesions of the small intestine and only rarely lesions of the large one.

Besredka attempted to obtain the toxin of the dysentery bacillus by pulverizing the killed and dried organisms with dry sodium chlorid, and subsequently dissolving them in distilled water. Di Donna also attempted to extract the toxin from the organism by the method of Lustig and Galeotti. By both these methods, however, probably only the endotoxin is obtained, and this not in a pure state.

The action of the toxin of the dysentery bacillus obtained by filtration of cultures is very evident when rabbits are inoculated intravenously. Severe diarrhea sometimes sets in, with paralysis of the limbs as well as of the muscles of the trunk and neck. The animal rapidly loses in weight and death occurs in from one to four or five days. When death occurs the lesions resemble those following the injection of the bacillus. Hyperemia and hemorrhages are often observed upon the serous membranes and upon the peritoneal surface of the intestine.

The toxin consists apparently of two components: first, a neurotoxin, which produces paralysis in the limbs and urinary bladder and sometimes hemorrhages in the cord and softening of the gray matter; and, second, the enteric toxin, which causes the dysenteric changes in the large intestine in man, and in animals in the small and sometimes the large intestine.

The variation of the susceptibility of different animals to the toxin is very striking, as was shown by Todd. The rabbit and horse are highly sensitive, the latter particularly so; while the guinea-pig, rat, mouse, and monkey are hardly affected. There seems to be a general agreement that the Flexner type of dysentery bacillus does not yield a soluble toxin.

As to the nature of the toxin of the dysentery bacillus, there has been much difference of opinion. A large number of the earlier investigators—Conradi, Neisser and Shiga, Vaillard and Dopter, Flexner and Sweet,
Ludke, Rosenthal, Todd, and Klein—regard it as an endotoxin. Arguments in favor of this view are that the toxin appears in greater quantities in older cultures in which already large numbers of the bacteria have become broken up, than it does in fresh ones; also, that, after the injection into animals of the dissolved toxin, exactly the same lesions are observed as follow the injection of the living or killed bacteria, or their extracts; also, that by immunizing animals with the whole bacillus a serum is obtained which in small quantities is capable of neutralizing the action of the detached toxin. An antitoxic serum produced with the detached toxin also contains agglutinins and bactericidal substances which are not different from those obtained in a serum prepared with the whole organism.

On the other hand, Kraus and Doerr, as well as Kolle, Heller, Neufeld, and others incline more to the view that the toxin is a true soluble one. The experiments of Flexner and Sweet do not conform with those of Kraus and Doerr, who stated that an active toxin could be obtained without autolysis. It seems evident, however, that the dysentery toxin, while not identical in action with the true soluble toxin of the diphtheria bacillus, nevertheless, resembles this toxin more than it does the toxin of either the typhoid bacillus or cholera spirillum. As was first shown by Todd, the antitoxic serum neutralizes the toxin in the test tube, and upon the subsequent injection of the mixture into rabbits no toxic action is observed. The serum also follows up to a certain extent Ehrlich's law of multiples.

It seems possible that the dysentery bacillus produces both a soluble toxin and in addition an endotoxin, which is more closely bound than the former. This fact, however, has not been conclusively demonstrated; but the production of an antitoxic serum is definite. Kraus and Doerr have emphasized as a further differentiation between the soluble toxin and the endotoxin of the dysentery bacillus that, after the injection of the endotoxin, as after inoculation with the dysentery bacillus in an animal, the symptoms of anaphylaxis may become evident, while after the injection of the dysentery toxin a specific susceptibility does not appear.

Ilorimi (1913) in studies carried on relating to the toxin of the dysentery bacillus, concludes that the neurotoxin, which is the main agent in producing death, is associated exclusively with living bacilli, its effects being most manifest when they are injected into the circulation. It may be washed out of the living bacillus along with the exotoxin by salt solution. The toxin washed out of either living or dead bacilli has a selective action on the cecum only. Repeated washings with salt solution of the same bacilli extract an endotoxin situated in the deeper layers of the organism, and this substance has a specific action on the small intestine. Final solution of the bacilli liberates a further substance to which the specific action on the colon is due. In bouillon cultures all of these substances appear to be present except the one which may be washed out
of either the living or dead bacilli, and which has a selective toxic action on the cecum.

DYSENTERY IMMUNE SERUM

**Bactericidal Reaction of Dysentery Immune Serum.**—Shiga first showed the bacteriolytic action of antidysenteric serum, demonstrating such action in vitro by the method of Neisser and Wechsberg, who found that a fixed amount of serum containing normal complement, which in itself was incapable of causing bacteriolysis, will destroy a given amount of a specific organism in the presence of a definite quantity of immune serum free from complement. The addition of an amount of immune serum greater or less than that exactly requisite will allow of more or less unrestricted growth of the organisms. The growth obtained in the instance of a similar quantity of immune serum is explained upon the basis of an insufficiency of bacteriolytic amboceptor, and in the instance of a surplus of immune serum, of an excess of amboceptor, through which the complement is deviated from the bacterial cells by union with free amboceptors. This bactericidal action of dysentery immune serum was also carefully confirmed by Gay, and later by Moses. A somewhat similar action of the serum may also be observed, though somewhat less satisfactorily, when the injection of the serum and organism is made into the abdominal cavity of a guinea-pig. However, the production of Pfeiffer's phenomenon is frequently not satisfactory. Baecher and Laub recommend the withdrawal of small quantities of the peritoneal exudate from the animal from time to time, and the inoculation of this material upon agar plate cultures. This method also does not always give satisfactory results.

**Antitoxic Action of Dysentery Immune Serum.**—Todd first demonstrated the antitoxic action of a dysentery immune serum. Horses were immunized with the toxin obtained after filtration by growing the organism in ordinary alkaline broth, as employed for the production of diphtheria toxin. These animals were found to be very susceptible to the action of the toxin. The immunization was begun by giving .5 c. c. of the toxin subcutaneously, and injections of increasing quantities were made every third day for a period of 6 weeks, when a dose of 150 c. c. was reached. After a dose of 2 c. c. of the toxin the horse showed a rise of temperature, and the next day a pronounced diarrhea appeared. Later on, after a dose of 8 c. c., diarrhea again appeared. Finally, however, upon the injection of 150 c. c., complete paralysis of the hind legs occurred and the animal had to be killed. Eventually, however, a horse was immunized, as large an amount as 400 c. c. of the toxin being injected before the animal was bled. Another horse was immunized with the bacillus alone; at first killed cultures were employed, and later living ones. The immune sera prepared in this manner were shown to be highly anti-
toxic. In the case of an animal immunized with the bacillus .001 c. c. of the serum was sufficient to protect a small rabbit against 20 minimal lethal doses of the toxin. Todd, moreover, showed that the toxin and antitoxin require a certain time for their combination in vitro, and this time is dependent upon the temperature, varying from less than 5 minutes at 37° C. to between 1 and 2 hours at 0° C. The antitoxin was shown to be capable of protecting animals either when mixed with the toxin or when given separately at another part of the body, either at the same time or shortly before or after the toxin. Todd's results have been confirmed by Ludke, Rosenthal, Kraus, Doerr, Vaillard, Dopter, Kolle, Heller, Neufeld, and Moses, all of these investigators showing the possibility of production of antitoxic dysenteric sera. The demonstration of the presence of the antitoxin in the sera has been made by mixing the toxin and serum containing antitoxin in the test tube before the injection into the animal, or also by the separate injection of the toxin and serum.

Up to the present time no one has apparently obtained a specific antitoxic serum against the Flexner bacillus or other acid strains investigated from this point of view. From the statements in the literature it seems probable that this strain produces no true soluble toxin, or such a toxin in only very minute amounts.

**Method of Testing Antitoxic Value of Dysentery Serum.**—For the purpose of testing the antitoxic value of an antidysenteric serum Shiga recommended experiments upon mice. Five times the lethal dose of the dysentery bacillus culture (.4 mg.) is mixed with several doses of the serum and injected into the peritoneal cavity of the animal. In the serum prepared by Shiga .0025 c. c. of serum protected the mice from the infection of 5 times the lethal dose of the organism. Kraus, Doerr, and Schottelius recommend rabbits for the purpose of determining the antitoxic value of the serum. According to Kraus and Doerr, rabbits of from 800 to 1,000 gm. in weight should be employed, and four times the lethal dose of dysentery toxin injected into the ear-vein, and at the same time the quantity of the dysentery immune serum injected into a vein of the other ear. According to these investigators only such sera should be used in the treatment of human cases of dysentery as will still protect the animal in doses of .1 c. c. Todd also employed the rabbit for testing the antitoxic power of the sera produced by him. Vaillard and Dopter and Kolle and Heller tested the value of the dysentery serum upon white mice. They employed the method of first mixing the toxin and antitoxin in vitro and injecting intraperitoneally a mixture of four times the fatal dose with the varying quantities of sera. Schottelius, however, found, on the contrary, that the results obtained with white mice were not sufficiently reliable, and employed rabbits of 2,000 gm. weight, inoculating a mixture of four times the fatal dose of toxin and various amounts of the serum, the mixtures of toxin and antitoxin having been placed for a half hour at
DYSENTERY IMMUNE SERUM

37° C. before the intravenous injection. The separate injection of the toxin and serum which has been employed by some other authors is, in the opinion of Schottelius, inadvisable on account of the susceptibility of the rabbit for the toxin, which may become quickly bound in vivo. Todd pointed out that the rapid fixation of dysenteric toxin by the tissues of the body is entirely similar to the fixation of tetanus toxin, as was shown by Dönitz. Moses also recommended the rabbit in preference to the mouse for testing the antitoxic value of dysenteric sera. His injections were made intravenously into the saphenous vein. Some of the sera obtained by him from the horse neutralized four times the fatal dose of toxin in amounts of .005 gm. Guinea-pigs have been found by all investigators to be unsatisfactory for testing the value of antidysenteric sera. They are frequently found to be insusceptible to the action of the toxin.

Instability of Antitoxin.—Kraus and Doerr have shown that the dysenteric antitoxin in contrast to the toxin shows very little resistance. In serum which had been stored for the course of a year a considerable loss of power was observed. It is, therefore, advisable to examine antitoxic dysenteric sera from time to time in regard to their value. The antitoxin is also very susceptible to heat, being destroyed at a temperature of 70° C. A neutral mixture of toxin and antitoxin becomes toxic after heating, which shows that the union is merely a chemical one and that a destruction of the toxin by the antitoxin has not taken place, a phenomenon which has been observed in relation to other toxins and antitoxins.

Production of Antidysenteric Sera.—For the production of antidysenteric sera for man the horse is the most favorable animal. Sera for the treatment of dysentery in man were first prepared by Shiga in Japan in 1898-1899, and by the author in 1900-1901, and were used with success in the treatment of this disease. Various methods of preparation of dysenteric sera have been described, the animals being inoculated either with killed cultures of the organism, or with filtrates of bouillon cultures, or extracts of the organism, or even with living cultures. Shiga, Kruse, Gay, and others have used killed cultures, performing the inoculations intravenously. The preparation of the sera by means of the living cultures has been recommended particularly by Todd, Kolle, Krumbein, and Schurmann.

Kraus and Doerr recommend the immunization of the horse with the toxin contained in bouillon culture filtrates, the subsequent injections being given after long intervals of time. When in the course of immunization larger amounts of the toxin are injected, they advise that the animal should be inoculated subcutaneously some days previously with from 80 to 100 c. c. of the antitoxic dysenteric serum, previously prepared in other animals, in order to avoid a severe reaction on the part of the horse.
Sera prepared by the above methods show not only antitoxic value, but have a bactericidal and agglutinating action upon the dysentery bacillus. Shiga showed that there was a suitable complement in the human body to complete the bactericidal action of the dysentery serum.

**SEUM TREATMENT IN MAN**

The serum prepared by Shiga was first used in Japan with very favorable results. If the injections were given in the first stages of the disease the unfavorable symptoms either soon ceased or became greatly ameliorated. Often, one or two days after the injection, the blood and mucus disappeared from the stools, pain and tenesmus ceased, and the patient appeared entirely well. If the serum was injected later in the disease, for example at the end of the first week, improvement of the symptoms usually occurred on the day following the injection, the number of stools markedly diminishing and the tenesmus decreasing. After a few days remarkable improvement was frequently seen. Recovery usually took place after a week.

The effect of the serum upon the temperature is frequently striking. The temperature may be subnormal on the day following the injection. In the ulcerative stage the action of the serum is not so favorable. In such cases, however, the fever, if present, is reduced, and the number of stools markedly diminished. The general condition also is improved.

Of the 510 cases treated by Shiga, 298 with serum and 212 with drugs, in the cases which received serum treatment the mortality was less than one-half (9-12 per cent.) of that observed in the cases which received medical treatment (22-26 per cent.). The course of the disease in the cases which received serum was also much shorter than in those which received medical treatment only. Shiga also prepared a polyvalent serum by alternate immunization of horses with the three different types of dysentery bacilli. With this serum he states much better results have been obtained.

A very large number of investigators have demonstrated the value of the serum treatment in the acute forms of the disease. In general it may be stated that the mortality in the acute cases treated with the serum varies between 2 and 5 per cent., as compared with 10 and 50 per cent. in those in which purely medical treatment has been employed. Rosenthal had only 8 deaths among 157 cases which received the serum, or 5.1 per cent., while the general mortality from this disease in the vicinity at that time was between 12 and 17.5 per cent. Laptesch found that by using from 20 to 40 c. c. of an antitoxic dysenteric serum the mortality was reduced from 10 to 15 per cent. in the non-serum-treated cases to 5 per cent. in those treated with serum. The action of the serum varies according to...
the type of bacillus causing the disease, and appears to be specific. If, as has been stated, the infecting strain possesses marked toxic properties, then when the corresponding antitoxic serum is employed favorable therapeu
tic results usually follow. Vaillard and Dopter obtained favorable results in the treatment of dysenteric cases in an insane asylum, the mortality being reduced by the serum treatment from 22 to 12 per cent. In the treatment by them of patients afflicted with the Shiga-Kruse bacil-
lus the mortality was reduced from 20 to 50 per cent. to 5 per cent. by the serum treatment.

In general, the cases infected with the Flexner strain of organism, according to the reports in the literature, seem to be less benefited than the cases which are due to the Shiga-Kruse strain. It has been shown that serum made with the Shiga-Kruse strain produces little effect in cases of dysentery caused by the Flexner one, and, vice versa, serum produced by the Flexner strain exerts no favorable influence on dysentery cases in which the Shiga-Kruse type is the infecting organism.

In addition to Shiga, Gay, Coyne and Auche, Ruffer, Willmore, and others have employed a polyvalent dysenteric serum which has shown good results.

Ruffer and Willmore showed that the Shiga-Kruse dysentery serum produced no favorable results in their cases, which owed their origin to the Tor I or Y type of dysentery bacillus.

While in the treatment of the summer diarrhea in this country the serum prepared by Flexner and his associates seemed at first to exert a favorable action, a more extensive investigation of the subject showed that, upon the whole, very little or no definitely beneficial result was obtained. Escherich also did not obtain favorable results in children. Ruffer and Willmore, after preparing a serum with the dysentery type Tor I, obtained very good results in individuals infected with this organism. They also prepared a polyvalent serum by immunizing horses with a large number of dysentery bacilli of the different types, and were able to reduce the mortality from 64.4 to 10.8 per cent. by means of such a serum. In pure infections with the bacillus El Tor type and the specific serum the morta
tility was reduced to even as low as 5.7 per cent. Willmore's studies made during the past year have shown that by means of the serum treat
tment the mortality against bacillary dysentery in Egypt has been greatly reduced from year to year, from 1909 to 1911. During the year 1911-
1912, however, no serum was available, and then the mortality from this disease rose to 70 per cent. He resumed the treatment in 1913 with serum, the mortality again falling to about 12 per cent.

Violle, during an epidemic in which he studied 104 cases, treated 80 with serum with excellent results, the doses used being from 20 to 120 c. c. There were no deaths among these cases. Among the remaining 24 cases not treated with serum there were 4 deaths.
Rogers in Calcutta, and Brau in Saigon, have also reported during the past year upon the favorable effect of serum treatment in this disease. Bahr (1914) treated 72 cases with a polyvalent antisyphilitic serum. None of these cases succumbed to the dysentery, while in 53 cases which received no serum the mortality was 13.2 per cent. The author states that in apparently hopeless cases the injection of from 50 to 70 c. c. of the serum in the first 24 hours after admission was followed by remarkable improvement. After such injections no deaths occurred in a series of 5 cases in whom the disease was of the severe type.

**Dosage.**—Shiga recommended that in mild cases the serum be injected once in a dose of 10 c. c. In cases of moderate severity, 2 doses of 10 c. c. at an interval of from 6 to 10 hours should be given, while in severe cases from 40 to 60 c. c. were recommended. The daily dose was not to exceed 20 c. c. Other observers have recommended somewhat larger doses of the serum at times. In this country a dose of 30 c. c. has been recommended frequently for adults, and 15 c. c. for children. The serum prepared by the Pasteur Institute and the Lister Institute is recommended to be given in 20 c. c. doses twice daily, and in very severe cases 4 times daily. Ruffer and Willmore have employed from 80 to 120 c. c. of the serum, giving the injections either intravenously or subcutaneously, the injections being sometimes repeated twice daily, or at longer intervals, as the patient’s condition demanded. As a rule no unfavorable results follow the injection of serum in man. Occasionally erythema, urticarial eruptions, pains in the joints, and sometimes fever follow the injections. If these symptoms are severe calcium chlorid in doses of 1 to 3 gm. may be administered.

Savage noted the occurrence of death from nephritis after massive doses of a polyvalent antisyphilitic serum in his cases in Egypt, and it was at first thought the serum was not wholly innocent in this connection. In dysentery cases with nephritis an increase in the albumin of the urine was also noted in patients who had received large doses of the serum. However, after an investigation of this question, it was shown that the increased albuminuria after the administration of serum was due to the rapid elimination of the latter, and not to any exacerbation of the existing nephritis.

**Vaccine Treatment in Dysentery**

But little use has been made of the vaccine treatment in acute dysentery, but during the past year some investigations have been carried on in regard to the vaccine treatment of subacute and chronic forms of this disease. Forster used for this purpose a vaccine consisting of a suspension of the killed dysentery bacilli suspended in normal saline solution to which .5 carabolic acid had been added. Gillett employed this method
REFERENCES

——. Compt. rend. soc. biol., 1908, T. 64, No. 24.
Duval and Bassett. Studies from the Rockefeller Institute, 1904, ii.
——. Schorer. Studies from the Rockefeller Institute, 1904, ii.
——. Studies from Rockefeller Institute, 1904, ii, 59a.
——. System of Medicine by Allbutt and Rolleston, 1912, ii, 489.
Forster. Ref., Dysenteries and Their Treatment by Rogers, London, 1914, 293.
BACILLARY DYSENTERY

—. Centralbl. f. Bakt., Orig., 1907, 1 Abt., xlix, No. 2.
—. Deutsch. med Woch., 1908, Jahrg. xxxiv, No. 19.
—. Arbeiten aus dem Institute zur Erforschung der Infektionskrankheiten in Bern, 1908, No. 1.
—. Ibid., 1906, xix, No. 30.
—. Deutsch. med. Woch., 1908, Jahrg. xxxiv, No. 27.
—. Ibid., 1901, xxvi, 370.
—. Ibid., 1903, Nos. 1, 3, 12.
—. Centralbl. f. Bakt., Orig., 1905, 1 Abt., xxxviii, No. 3; ibid., xxxix, Nos. 5 and 6; 1906, xl, Nos. 1, 3, 4.
—. Deutsch. med. Woch., 1903, 6.
—. Ibid., 1911, 1, Beiheft, 142.
REFERENCES

—. Arch. f. Schiff- u. trop. Hyg., 1914, xviii, 259.
Rogers. Dysenteries, Their Differentiation and Treatment, London, 1913, 290.
—. Deutsch. med. Woch., 1903, No. 6.
—. Ibid., 1904, Nos. 7 and 19.
—. Ebenda, 1 Nov., Orig.-Ref. in Centralbl. f. Bakt., 1 Abt., Ref., 1904, xxxiv, Nos. 16 and 17.
—. Ibid., 1909, ii, 462.
—. Ibid., 1910.
—. Deutsch. med. Woch., 1903, No. 7.
—. Phila. Jour. of Sci., 1906, i, No. 5.
—. Modern Medicine by Osler and McCrae, 1913, i, 766.
Todd. Jour. of Hyg., 1904, iv, 480.
Vaillard and Dopter. Presse méd., 1903.
—. Bull. de l’acad. de méd., 1906, Sér. 3, lv, 265.
—. Bull. de l’acad. de méd., 1907, Sér. 3, T. lvii, No. 15.
—. Presse méd., 1907, No. 45.
CHAPTER IX

PLAGUE

RICHARD P. STRONG

VACCINE THERAPY

While vaccination against bubonic plague as a prophylactic measure has been extensively employed with results warranting its use, no practical application has been made of vaccine treatment in plague. The course of the disease is too acute for such a measure to yield satisfactory results, since the majority of cases die in from three to five days after the onset of symptoms. The serum treatment in plague, however, has been extensively employed.

SERUM THERAPY

Specific Immunizing Properties of the Serum.—In order to have a proper understanding of the serum treatment of plague and of its value it is necessary to be familiar with the action which the plague immune serum exerts upon the plague bacillus in the animal body, and the manner in which it destroys it. The mechanism by which the plague bacillus is rendered innocuous by such a serum is quite different from that by which, for example, the cholera organism is destroyed by cholera immune serum or the toxin of the diphtheria bacillus acted upon by antitoxic diphtheria serum.

Bactericidal Reaction.—Early investigations seemed to suggest that the plague immune serum exerted a bactericidal effect. Pfeiffer and Dieudonné, of the German Plague Commission, concluded that in plague immune sera specific bactericidal antibodies were present, the action of which was fully analogous to that of the protective substances which had been demonstrated to exist in cholera and typhoid immune sera. Apparently no experiments were made which demonstrated that the plague serum possessed a bactericidal action, although some experiments were performed which demonstrated its preventive action against infection and its curative value. For a time the opinion that plague immune serum exerted a bactericidal action against the plague bacillus became generally
accepted, although but little experimental work was carried on upon
the subject. Kolle and Martini performed experiments with guinea pigs
and rats, in which the animals were inoculated with from 1 to 2 c. c. of
plague immune serum and 24 hours later were inoculated intraperitone-
ally with from 2 to 3 loops of plague cultures of moderate virulence,
suspended in saline solution. Upon microscopical examination of drops
of the exudate from the abdominal cavity 3 or 4 hours after the inoculation
of the bacteria, the majority of the bacilli were found to be swollen,
degenerated, and broken up. This phenomenon was not noted in control
animals treated with normal serum, but plague bacilli of normal appear-
ance were observed, which increased in number from hour to hour up to
the time of the death of the animal. The abdominal exudate of the
animals treated with the immune serum was sometimes apparently sterile
after 24 hours, although in these cases the few remaining bacteria actually
present usually multiplied and caused the death of the animal at a later
time. Rats which had been previously actively immunized against plague
by repeated subcutaneous injections of plague cultures, when inoculated
intraperitoneally with plague strains of moderate virulence also exhibited
the same bactericidal action toward the bacteria. No antitoxic action
could be observed. Markl found that the method of destruction of plague
bacilli varied according to the virulence of the organism. When a culture
of very great virulence was inoculated into the abdominal cavity of a
guinea-pig which had been treated with an immune serum after 30
minutes a very extensive leukocytosis occurred, and the bacteria were
taken up by the phagocytes. Those bacteria which remained free became
agglutinated and grouped about the leukocytes. One hour after the injec-
tion of the immune serum no extracellular bacilli could be found in the
peritoneal exudate, and cultures made from it either remained sterile or
only a few colonies developed. A leukocytosis also occurred in control
animals without immune serum, but the bacilli remained in this instance
extracellular, and cultures of the abdominal exudate on agar produced a
rich growth of bacteria. In other experiments on rats the very virulent
strains of the plague organism were taken up by the phagocytes through
the action of the immune serum, while the avirulent strains, those non-
lethal in doses of two loops, became dissolved in the abdominal cavity
of the animals without the aid of the phagocytes. Strains of moderate
virulence were partially destroyed in both of these ways. The same
mechanism was observed in passive immunization with serum as was
seen in animals actively immunized either with killed or with living at-
tenuated cultures. Skschivan also obtained Pfeiffer's phenomenon in
guinea-pigs which were inoculated with 4 c. c. of the Paris plague immune
serum, and 16 to 20 hours later were reinoculated with from two loops up
to one agar culture intraperitoneally. When the serum was inoculated into
the peritoneal cavity the bacteria became broken up in one-half hour.
The control animals without serum died after one to two days, while those inoculated with immune serum lived for from 5 to 7 days.

**Anti-infections or Antibacterial and Opsonic Action.**—Later more complete and carefully controlled experiments performed by Kolle and the writer showed that the plague immune serum exerts no other demonstrable and typical bactericidal reaction against the virulent plague organism during the course of an infection than a normal serum. The method of action of plague, cholera, and typhoid immune sera was compared, the bactericidal action being tested in vitro after the method of Neisser and Wechsberg. In spite of many variations in the experiments and in the use of many different sera from different species of animals to supply the complement for the action of the amboceptors, plague bacilli after treatment with the plague immune serum developed as plentifully in the culture media as they did in those instances in which they were treated with normal sera.

In studying the bactericidal action of plague immune serum, both inactivated serum, to which fresh serum was added to supply the complement, and plague immune serum perfectly fresh and not inactivated were experimented with. When perfectly fresh sera are employed in these tests it is true that both the normal serum and the plague immune serum exert a lytic effect upon the plague organism; this action appears to depend upon the presence of fresh complement, as it can be abolished by heating the serum previously at 55° C. for one-half hour. It, however, does not interfere in estimating the bactericidal effect of plague immune serum as compared with that of normal serum. A plague immune serum from the horse, not inactivated, which at the time of the experiment in doses of 1 c. c. was able to protect about 90 per cent. of the rats inoculated with it against fatal plague infection, was mixed with perfectly fresh rat serum, and its bactericidal value tested according to the usual method in vitro. In order that the phenomenon of the deflection of the complement by amboceptors might not interfere with the reaction the experiments were also performed with varying amounts of the immune horse serum and fresh rat serum. However, again no differences could be detected between the results obtained with these experiments and with those performed in the same manner with normal horse serum to which fresh rat serum had been added.

These experiments appear to demonstrate that the plague immune serum, which is known to possess immunizing power in the animal and which prevents the further development of the infection, possesses in vitro no bactericidal action whatever, that is, similar to that exerted, for example, by typhoid immune serum. It is also clear that the plague bacilli are not only not killed by the immune serum in vitro, but that they remain alive and are capable of subsequent development. Therefore, some other factor must play an important rôle in the ultimate destruction of
the inoculated bacilli in the body of an animal passively immunized by
the injection of such a serum, and, since the serum alone in the test tube
apparently exerts no marked injurious action upon the plague bacilli, it
appears that the phagocyte is the additional factor which is necessary to
render harmless and to destroy the organism in question.

In elucidating this question it is advisable to consider not only what
action the serum has upon the life of the plague organism, but also what
action the organism has upon the immune serum. We know that when
the specific substances of a serum such as antitoxin or bacteriolysin are
brought into contact in vitro with the homologous bacterial antigen a union
occurs between them. Although the union between these two substances
follows a different law, it is possible to show that such a binding actually
does take place, and that the antitoxic serum loses in value after combina-
tion with toxin and the bactericidal one diminishes in its specific effect
after treatment with the corresponding bacterium. In order to understand
this relationship between the plague bacillus and its corresponding immune
serum, a plague immune serum was first carefully tested for its immuniz-
ing power on rats, and the amount determined which would protect about
90 per cent. of the animals inoculated with it against the subsequent in-
jection of a lethal dose of plague bacilli. Fifteen c. c. of this plague
serum were then mixed with the living bacteria obtained from fifteen
48-hour agar slant cultures of a virulent plague organism. The mixture
was placed in the incubator for two hours at 37° C. Carbolic acid to .5
per cent. was then added to the mixture which was next heated for 2
hours at 46° C. and finally thoroughly centrifuged. The clear fluid above
was then drawn off from the sediment of bacteria. After the sterility of
the serum had been demonstrated its immunizing value was now for a
second time tested on rats, and it was then found that the serum no longer
protected these animals in the same amounts as it did previous to its
treatment with the bacteria, 70 per cent. of the rats inoculated with the
same dose succumbing when subsequently infected with plague. From
this experiment it is clear that a binding of at least a portion of the ambo-
ceptors of the plague immune serum to the receptors of the plague bacillus
had occurred, and, although the bacteria in question were not killed by
the serum, nevertheless a reaction in vitro between the serum and the
organism had occurred.

For the further study of the action of plague immune serum other
experiments were performed in vivo in the abdominal cavities of guinea-
pigs. Upon injecting a virulent plague organism into the peritoneal cavity
of a guinea-pig temporarily immunized by the injection of plague immune
serum it was found that Pfeiffer's phenomenon, as observed in the case
of the cholera organism in the cholera-immune animal, did not occur, the
virulent organism in question did not undergo dissolution, and only when
very avirulent strains of plague were employed did the organisms finally
become swollen or disintegrated. This latter observation explains the previous results obtained upon this subject. It is true that shortly after the inoculation of the virulent plague strain in the immunized animal a disappearance of the bacteria from the abdominal cavity usually occurs, and that also at first but few animal cells are encountered in the abdominal exudate. Upon investigating the fate of the bacteria by killing animals at different periods of time after the inoculation it was found that shortly after the injection, both in the case of animals immunized against plague and in that of normal animals, the bacteria had been carried to, or made their way to, the cells of the cavity, and particularly to the omentum, to the surface of which they had become adherent. Here many of them were taken up by the phagocytic cells. After a short period the leukocytes became more abundant in the abdominal exudate and many of them were seen to contain bacteria. In many cases in the immunized animal the leukocytes seemed to possess positive chemotaxis for the bacteria, judging from the manner in which the latter were grouped about them. In the case of non-immune animals the plague bacilli outside of the cells increase in number up to the time of the death of the animal. The majority of the bacteria that are found to exist free in the cavity after the short period of their disappearance are short, bipolar, staining bacilli which often seem to possess capsules. A small number of large bacilli, frequently showing involution forms, are also encountered. After the temporary disappearance of the bacteria in the case of the immunized animal, the leukocytes usually become much more numerous in the abdominal cavity.

The phagocytosis of the bacteria continues both by the cells in the omentum and by those free in the abdominal cavity until very few free bacilli remain. However, in the non-immune animals the bipolar staining organisms, which increase up to the time of the death of the guinea-pig, do not appear to be taken up by the leukocytes. It would appear that the phagocyte usually ingests only the organisms which have previously been affected by the immune serum.

From what has been said it is obvious that when plague immune serum is brought into contact with the plague bacillus in the test tube the amboceptors of the serum unite with the receptors of the organism and that in the body of the animal the process of destruction is carried on further by the leukocytes which engulf the bacteria which have been so acted upon. It is also evident that the bacteria are not killed in the test tube by the immune serum alone. It appears that, after the bacillus has been prepared for the action of the leukocyte by the immune serum, the latter plays a part in the digestion and ultimate destruction of the organism. This destruction, however, does not always, at least, seem to occur immediately, since, when loops of the abdominal exudate which contain phagocytes enclosing plague bacteria are transplanted to the surface of agar, the
organisms under these circumstances sometimes increase within the cells and in some instances burst the leukocyte and partially escape from it.

The destruction of the plague bacillus is therefore effected by the immune animal in a manner partly in accord with the humoral theory of Buchner, and partly in accord with the phagocytic one of Metchnikoff. The action of the serum in its protective effect upon the animal is neither antitoxic nor bactericidal, but may be termed as anti-infectious or antibacterial, that is, it is a serum possessed with the power of preventing infection, and from the rôle already described which the phagocytes play in the process, its action may also be said to be opsonic in nature. It also has been demonstrated that the opsonic index of a plague immune serum is higher than that of a normal serum.

Rowland in studying recently the action of plague-immune serum arrives at practically the same conclusions which have been just stated, and believes that the essential factor in plague immunity is one which affects the multiplication of the bacillus. In his experiments he was able to show that in the immune animal the multiplication of the inoculated plague bacilli is much less than in the case of the normal animal. In the abdominal cavity of the guinea-pig the bacteria were observed inextricably entangled in a mass of fibrin and cells. Many of the cells were filled to bursting point with the bacteria. The fate of the animal seemed to depend upon the rate of the engulfing of the micro-organisms by the cells within a mass of fibrin, and the rate of multiplication of the bacteria. If the rate of the engulfing competes successfully with the rate of multiplication, then the animal survives. If, on the other hand, the rate of multiplication of the bacilli is greater than the mechanism of engulfing, phagocytosis, and lysis can compete with, then the animal succumbs to plague. In the immune animal he found there were finally no free bacilli. In the normal and immune animals the difference in the reaction seemed to depend more upon the quantity of bacilli present than on anything else. The number of bacilli in the case of the immune animal was at any stage of the process much less than was the number at the same stage in the case of the non-immune animal. In the subcutaneous inoculation of immune and non-immune animals he also came to the same conclusion, namely, that the essential factor in plague immunity is one which affects the multiplication of the bacillus.

Result of Treatment in Animals.—Bearing these phenomena in mind in relation to the mechanism of the action of plague immune serum, it is not difficult to interpret the results which are obtained in the serum treatment of animals experimentally infected with plague, and we find that the success of the serum treatment appears to depend particularly upon the number of plague bacilli in the animal organism at the time of the inoculation of the serum, that is, upon the length of time the serum is injected after the infection has occurred. If the organism is already
overwhelmed with bacteria at the time of the introduction of the serum, almost no favorable change will be noted in the course of the disease, because the serum is merely anti-infectious and is not antitoxic.

Thus, of a series of rats inoculated with immune serum at the time of their infection with plague bacilli, 60 per cent. survived and 40 per cent. succumbed to the infection; while of another series which were inoculated with the serum 24 hours after the plague infection only 40 per cent. survived and 60 per cent. died. In another series of experiments in which larger doses of serum were employed, and a less severe method of infection, the animals were inoculated with the serum in three series; one at the time of the infection, a second 24 hours following the infection, and a third 48 hours after the infection. The mortality in the first series was 10 per cent.; in the second 40 per cent.; and in the third 66.6 per cent. Similar results have been obtained with monkeys, and sometimes it is possible to save those animals which have previously been infected with plague by the inoculation of plague immune serum injected as late as from 12 to 24 hours after the time of the infection, provided large doses of the serum are used. With rats it has been shown that if large doses of the serum are used, even animals in which the disease is fairly well advanced may sometimes be saved by the serum.

Result of Treatment in Man.—Turning our attention to the treatment of human cases of plague with serum, we find somewhat similar results. Choksy, who has had the most extensive experience with the serum treatment of plague, states that much depends upon the early and free use of the serum. In patients treated on the first day or within a few hours of the onset of the symptoms one injection of 100 c. c., followed by another after 6 to 8 hours, and then, if necessary, by a third after a similar interval, would cut short the attack if the case were not pneumonic, malignant, or septicemic. He also emphasizes the fact that the earlier the serum is used the more efficacious it is, and that, if good results are to be obtained from serum therapy, the patient must be treated on the first day of the illness. He admits that the serum cannot favorably influence all types of plague, or even the malignant forms of the bubonic type, but he shows that it is the only treatment capable of saving a large proportion in a certain class of patients.

In his last publication regarding the subject he summarizes observations regarding 1,081 cases. There were eliminated from the observations septicemic, pneumonic, and moribund cases, as well as convalescent and semi-convalescent cases, and also those in whom the illness had already lasted for six days or more. The observations were thus restricted to the most acute cases within the first five days of the illness. Every alternate case was then treated with serum. Four hundred cases under the observation of the author were treated in this way. In the serum cases the mortality was 63.5 per cent., and in the 200 controls the mortality was
74 per cent. There was thus a difference of 10.5 per cent. in favor of the serum cases. In a previous series of 238 cases treated with the serum, the mortality rate was 59.2 per cent. By comparing the time of death after admission between the serum and the control cases, it was found that, whereas 79 per cent. of all deaths among controls occurred within 4 days after admission, the proportion was 58.2 per cent. among the serum cases, a differences of nearly 21 per cent., the serum having considerably prolonged life. Of 243 cases treated in private practice with the serum, the mortality was as low as 40.7 per cent.

Out of the entire 1,081 patients subjected to the serum treatment 537 died and 544 recovered, the mortality rate being 49.6 per cent.; 613 of the cases were treated in hospitals in which the case mortality was 57 per cent., and 468 were private cases in which the mortality was 39.9 per cent. A very striking feature is the difference in the mortality rate according to the stage of the disease at which the serum was injected. Of 316 patients treated on the first day 220 recovered, the mortality being 30.3 per cent. On the second day of illness 300 cases were treated, 142 recovering, or a mortality of 52.6 per cent. The following table also shows the increased mortality in the cases treated later than the second day of the disease:

<table>
<thead>
<tr>
<th>Duration of Illness</th>
<th>Number</th>
<th>Recovered</th>
<th>Case Mortality Per Cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>First day</td>
<td>316</td>
<td>220</td>
<td>30.3</td>
</tr>
<tr>
<td>Second day</td>
<td>300</td>
<td>142</td>
<td>52.6</td>
</tr>
<tr>
<td>Third day</td>
<td>246</td>
<td>91</td>
<td>63.0</td>
</tr>
<tr>
<td>Fourth day</td>
<td>105</td>
<td>45</td>
<td>57.1</td>
</tr>
<tr>
<td>Fifth day</td>
<td>52</td>
<td>20</td>
<td>61.5</td>
</tr>
<tr>
<td>Sixth day</td>
<td>14</td>
<td>6</td>
<td>57.1</td>
</tr>
<tr>
<td>Seventh day</td>
<td>4</td>
<td>0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

The general mortality of plague at that time in India was estimated at 89.9 per cent. The author concludes his observations by stating that the success of the treatment lies in applying the serum very early. Among patients subjected to the treatment within the first few or even 24 hours it is noticed that the whole course of the disease becomes altered. The normal duration of the disease from about 8 to 10 days is reduced to 4 or 5 days. Serious complications of the nervous, circulatory, and other systems are averted. The buboes become absorbed, and convalescence is more rapid. After 48 hours the serum does not appear to influence the course of the disease perceptibly.

Simpson, in his "Treatise on Plague," summarizes his remarks in regard to treatment with the statement that if the serum is injected intravenously and early, it appears to give the patient a better chance of recovery than any pharmacopeial drug, and in some instances the state of
the patient after the injection is so much improved that it can only be attributed to the action of the serum.

Kitasato states that the good results obtained from the serum treatment admit of no dispute, provided sufficient quantities are used, 200 to 400 c. c., and that, although we are not in a position to ascribe to the pest serum a value as absolute as to the diphtheria serum, there is no doubt of the efficacy of the former remedy. A series of experiments was conducted by him in Formosa with a view to comparing the results of the serum with those of an early extirpation of the buboes and general systematic treatment. Of the 56 patients treated by the latter method 35 (62.5 per cent.) died of plague; while out of the same number inoculated with serum the death rate was only 33.9 per cent.

Burnett in his report of plague in Queensland has also obtained favorable results in the serum treatment of plague. From 1900-1907 300 cases were observed. The mortality in the cases treated with serum was 29.7 per cent., and the mortality of those who received no serum was 73.9 per cent., as may be seen from the following table:

<table>
<thead>
<tr>
<th>Year</th>
<th>Entire Number of Cases</th>
<th>Fatal Cases</th>
<th>Mortality—Per cent</th>
<th>Number</th>
<th>Fatal Cases</th>
<th>Mortality—Per cent</th>
<th>Number</th>
<th>Fatal Cases</th>
<th>Mortality—Per cent</th>
<th>Difference in Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1900</td>
<td>56</td>
<td>25</td>
<td>44.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1901</td>
<td>36</td>
<td>12</td>
<td>33.3</td>
<td>28</td>
<td>6</td>
<td>21.4</td>
<td>8</td>
<td>6</td>
<td>75.0</td>
<td>53.6</td>
</tr>
<tr>
<td>1902</td>
<td>82</td>
<td>26</td>
<td>31.7</td>
<td>69</td>
<td>15</td>
<td>21.7</td>
<td>13</td>
<td>11</td>
<td>84.6</td>
<td>69.2</td>
</tr>
<tr>
<td>1903</td>
<td>21</td>
<td>11</td>
<td>52.3</td>
<td>16</td>
<td>7</td>
<td>43.7</td>
<td>5</td>
<td>4</td>
<td>80.0</td>
<td>36.3</td>
</tr>
<tr>
<td>1904</td>
<td>30</td>
<td>8</td>
<td>26.6</td>
<td>25</td>
<td>6</td>
<td>24.0</td>
<td>5</td>
<td>2</td>
<td>40.0</td>
<td>16.0</td>
</tr>
<tr>
<td>1905</td>
<td>28</td>
<td>15</td>
<td>53.5</td>
<td>21</td>
<td>10</td>
<td>47.6</td>
<td>7</td>
<td>5</td>
<td>71.4</td>
<td>22.8</td>
</tr>
<tr>
<td>1906</td>
<td>11</td>
<td>7</td>
<td>63.3</td>
<td>7</td>
<td>4</td>
<td>57.0</td>
<td>4</td>
<td>3</td>
<td>75.0</td>
<td>18.0</td>
</tr>
<tr>
<td>1907</td>
<td>36</td>
<td>14</td>
<td>39.0</td>
<td>32</td>
<td>11</td>
<td>34.0</td>
<td>4</td>
<td>3</td>
<td>75.0</td>
<td>40.6</td>
</tr>
</tbody>
</table>

|        | Total                   |             |                     | 300    | 118         | 39.3                | 198    | 59          | 29.7                | 73.9                   |

D'Hospitalrich has also recently reported upon serum treatment of plague in Annam. Of 232 cases under the care of this author 21 were treated symptomatically, only 6 of whom recovered, a mortality of 97.5 per cent. Of 190 patients who received daily subcutaneous injections of 40 to 80 c. c. of Yersin’s antiplaque serum 128 died, or a mortality of 67.7 per cent. In 16 patients who were suffering from very severe infection large doses of serum up to 100 c. c. were injected intravenously; 4 of these survived. In 5 very severe cases intravenous injections of saline solution and serum in large amounts were given; 3 of these recovered. Of 9 serious cases in which the serum was given within the first 48 hours
of the illness 4 recovered. The author believes the serum exerts a beneficial effect if its administration is begun soon after the onset of the disease. If it is delayed to the third day or later, no favorable results are usually obtained.

In 1913 the British Commission published the results of a further study upon a large number of cases in Indiâ, in which the serum treatment was employed. In all 444 cases were observed, 222 being treated with serum and the remaining number serving as controls. Every alternate case in the hospital received serum, the moribund and those who had almost recovered alone being excluded from consideration. A few cubic centimeters of blood were aseptically taken from a vein of each selected case. One-fourth of a cubic centimeter was spread over the surface of an agar tube, and after incubation for 48 hours the cultures were examined. The cases were thus divided into 4 groups. In the first group the cases with no septicemia were classified, and the remaining cases were placed in the 2d, 3d, and 4th groups, according to the degree of septicemia present at the time. Two kinds of serum were used: first, the ordinary Yersin serum, prepared at the Lister Institute, London, by the injection of dead and afterward living bacilli; second, a serum prepared from horses injected with a toxic nucleoprotein which it is stated was efficacious in protecting rats from the injection of living broth cultures of plague bacilli. The amount of serum which gave such protection is not stated, nor is the anti-infectious power of the Yersin serum given. The serum was given in large doses, generally both intravenously and subcutaneously. Sometimes it was given subcutaneously only, and in a few cases intravenously only. In many cases further doses were given, usually subcutaneously on succeeding days. The majority of the patients received over 100 c. c. intravenously and some of the patients received altogether 500 c. c. of serum, both by subcutaneous and intravenous injections. Grouping all of the cases together, those with well-marked septicemia, as well as those with no septicemia, at the time of beginning the treatment, it was found that the mortality in the treated cases was 66.2 per cent., and in the cases untreated with serum 73.9 per cent. One hundred and forty-seven of the cases treated with serum died, and 164 of the controls without serum died; seventeen of the cases being saved by the serum.

The Commission conclude from their inquiry that it appears that the administration of the available sera is not a practicable means of bringing about any material diminution in the mortality of plague in India. This conclusion seems justified from the statistics which they have compiled after consideration of both the septicemic and non-septicemic cases together, and for the sera employed. The necessity of giving the serum early in the disease if any beneficial effect is to be expected has already been emphasized in this article, and in regard to this point the
Commission add to their conclusion the statement that it may well be that better results would be obtained if the treatment could be commenced within a few hours of the onset of the disease. When one analyzes the statistics obtained by them it may be seen, however, that the results are not so divergent from those which have been obtained by some other observers.

In the cases with no septicemia, Group I, there were 70 control cases, 24 of whom died, or 34 per cent.; while of 85 cases which received serum treatment only 22 died, or but 26 per cent. It is unfortunate that in this series there were not as many control cases without serum as there were cases treated with serum. A mortality of 34 per cent. is unusually low for plague, and possibly if a comparison had been made with an equal number of controls, more of the additional cases would have developed septicemia and succumbed.

In the study of their tables a perhaps still more striking feature is developed. Of 8 cases treated with the Yersin serum on the first day of the disease before septicemia had developed all recovered. These were the only cases of this nature which were treated with Yersin serum. In India, as the statistics show, the majority of the patients are not brought to the hospital before the second day of the disease, and, as we have already emphasized, but little benefit can be expected from the serum treatment of plague unless the serum is employed before this time. Of the 24 cases which they treated with Yersin serum on the second day of the disease before septicemia had developed 17 recovered and 7 died, a mortality of 29 per cent., while of 24 control cases not given serum who entered the hospital on the second day of the disease and before septicemia had developed 10 died, a mortality of 41.6 per cent.

The results, therefore, seem to show, as the others related have, that if the serum can be given early enough in the disease, and if the infection is not too severe, a beneficial effect may be often obtained. The result of serum treatment in plague, however, is frequently uncertain, and it must be borne in mind that it is only within a narrow limit of time that its use in man as in animals is efficacious.

**Treatment in Pneumonic Plague.**—In the treatment of pneumonic plague, however, serum treatment has given no favorable results, and it can only be stated that the serum in some instances has appeared to have prolonged somewhat the life of the patient.

In the early stages of the disease the serum appears to cause a fall in temperature and a temporary improvement in the general condition of the patient. During the recent Manchurian epidemic the fall in temperature usually occurred during the first three hours after the injection, and lasted for from 6 to 12 hours. Sometimes the temperature fell from .5 to 2.5° C. after the injection. After the fall, the temperature usually again suddenly rose. Sometimes following the injection the pulse
became stronger. The injection of serum did not prevent the development or extension of the pneumonia to other lobes of the lungs unaffected at the time of the injection, nor did it prevent the development of septicemia. After septicemia had developed the serum seemed to exert no favorable effect whatever upon the patient. Only when given in a very early stage of the disease did it appear to prolong the illness.

Of 42 human cases of pneumonic plague treated with antiplague serum during the recent Manchurian epidemic 33 received the first injection of serum within 6 hours after the first symptoms of the illness had appeared. The remaining 9 received injections of serum on the second day of the disease. All of them died of pneumonic plague. The injections were given both intravenously and subcutaneously. No difference in the course of the disease was observed with either of these methods. The quantities of serum injected varied from 100 to 1,700 c. c. All of the cases which were treated with serum died, so far as is known, with the exception of three cases reported from Dahny, but in these three cases the International Plague Conference considered that the bacteriological diagnosis of the disease was not sufficiently definite. The general experience throughout the epidemic, therefore, was that no method of treatment was of any value in saving life, and that the serum treatment seemed only in a few instances to have prolonged the duration of the illness.

Selection of Serum. — In employing serum in the treatment of plague the physician should be sure that the preparation is a reliable one. Plague immune sera have sometimes been offered for sale in which the immunizing power is so small as to render them practically of no value in the treatment of the human disease. The preparation of a satisfactory plague immune serum is tedious, difficult, and expensive, since it requires a long period of time to successfully immunize the horse from which the serum is obtained, and the animal not infrequently dies during the course of such immunization.

Method of Testing the Immunizing Value of Serum. — Before using a serum in an epidemic of human plague it is well to have its immunizing power tested upon rats in the following manner: The doses of the diluted serum should be injected intraperitoneally, a blunt syringe needle being employed for the injections, and immediately after the rat should be inoculated with a 5 c. c. syringe needle dipped in a suspension of plague bacilli in bouillon (one 48-hour agar culture of a virulent organism to 5 c. c. of bouillon), the needle being thrust under the skin for its full length near the root of the tail and then withdrawn. The serum should, of course, be inoculated in various amounts, and the experiment should always be performed in duplicate or triplicate, 2 or 3 animals being employed for each dose of serum, and an equal number of controls. A good plague immune serum should save from fatal infection at least 50 per cent. of the inoculated rats.
Varieties of Sera.—The variety of plague immune serum which is generally used is prepared from the horse by first the inoculation of killed cultures of the plague organism, and later by the inoculation of increasing amounts of living virulent organisms, and usually by filtrates of old bouillon cultures. A serum prepared in this manner is often spoken of as Yersin serum. Sera of practically this nature are prepared at the Pasteur Institute in Paris, and by the Institute for Infectious Diseases in Bern. Sera obtained in this way are at the present time generally acknowledged to possess the highest immunizing value. The method of preparation may be shortened by beginning with living avirulent cultures in place of killed ones. Another plague immune serum has been prepared after the method of Lustig and Galeotti in which the nucleoprotein of the plague bacillus is inoculated subcutaneously and intravenously into the horse furnishing the serum. Terni described a method of preparing an antiplague serum which he believed was especially active against the plague toxin. The animal furnishing the serum was inoculated with peritoneal exudates from guinea-pigs dead of plague and with the serum from plague buboes. Terni believed that these exudates contained aggressin. However, the writer has shown that the immunity obtained by the injection of natural plague aggressin is not of a different nature (so far as it concerns specific immunization) from that secured by the inoculation of living plague cultures, and hence the serum prepared in this manner has no advantage over one prepared by the inoculation of living organisms, as the results in man have shown. Of 111 cases treated with Terni's serum the mortality was 81.08 per cent., while of 112 parallel cases receiving no serum the mortality was 81.25 per cent.

Antitoxic Sera.—The plague toxin is an endotoxin. It differs somewhat from the toxin of the cholera or typhoid organism in that it becomes more easily set free from the bodies of the bacteria, but so far it has not been possible to prepare a satisfactory antitoxic plague serum for treatment.

Markl, Dean, Rowland and MacConkey have experimented with the idea of obtaining antitoxic plague sera either by using for the inoculation of the animal filtrates from old bouillon cultures, or by extracting toxins from the plague bacillus. So far these sera have not shown any advantage over those prepared by the usual method already described.

Rowland has prepared a serum in horses by inoculation of a nucleoprotein which he has obtained from the plague bacillus by a method which he characterizes as a sulphating process, dilute sodium sulphate or salt solution being used for its extraction. This serum was employed in India in 1913 for the treatment of human cases, but also showed no superiority over the Yersin plague serum.

Multivalent Serum.—Hetsch and Rimpan have performed experiments in preparing a multivalent plague immune serum, using 19 dif-
ferent strains of the organism for the purpose. The value of such a
serum was afterward tested upon rats. It was shown, however, that such
a polyvalent serum possessed no advantages over a univalent one. The
plague immune serum produced with one satisfactory plague strain will
exert its anti-infectious action against all strains of the plague bacillus, no
matter what their source, hence a plague polyvalent serum is not more
or less effective in its action against any one of these different strains than
is a univalent one.

REFERENCES

British Commission. Seventh Report, on Plague Investigations in India,
Jour. of Hyg., Plague Supplement II, Jan., 1913, 326.
Dean. Studies in Pathology written by the Alumni to Celebrate the
Quarter-centenary of the University of Aberdeen, Aberdeen, 1906.
———. Ibid., 1903, xlii, 244.
Rowland. Jour. of Hyg., Plague Supplement II, Jan. 1913, xii, 340,
358, 367.
———. Ibid., Plague Supplement III, Jan., 1914, xiii, 403, 412.
———. Ibid., Plague Supplement I, 1912, xi, 11.
———. Ibid., 1907, ii, Sec. B, 155.
Terni and Bandi. Deutsch. med. Woch., 1900, xxvi, 463.
CHAPTER X

ASIATIC CHOLERA

RICHARD P. STRONG

VACCINE TREATMENT

Vaccination against cholera has given sufficiently favorable results to warrant its further and more extensive use as a prophylactic measure, but owing to the extremely acute nature of the disease vaccine treatment in cholera is of no value. The most acute symptoms of intoxication occur within from a few hours to 2 or 3 days of the onset of the disease.

SERUM TREATMENT

The serum treatment so far on the whole has been very unsatisfactory. Indeed, several recent text-books upon medicine either fail to mention it or dismiss the subject with the statement that such treatment is of little therapeutic value. It, however, is advisable for the physician and investigator to understand the manner in which the various sera which have been prepared for treatment in this disease exert their action upon the cholera organism and its toxin in order to realize just how much is to be expected of these different sera in the way of treatment.

The Cholera Toxin.—In regard to the exact nature of the cholera toxin, and as to whether the organism produces a true soluble toxin, similar to that, for example, of the diphtheria bacillus, there is still considerable difference of opinion. Many observers have insisted that all the pathological changes brought about in Asiatic cholera are due to the action of an endotoxin. So far it has not been demonstrated that the organism produces a soluble toxin similar to that produced by the diphtheria bacillus, and it has not yet been possible to produce a pure antitoxic serum which neutralizes toxin and which at the same time follows Ehrlich’s law of multiples. Extensive studies, however, have been carried on in relation to the discovery of an antitoxic serum. The toxin of the cholera organism seems to exist as the constituent of the cell, or as an endotoxin,
SERUM TREATMENT

and to become soluble only through the disintegration of the organism. This was the original view of Pfeiffer, and, while his earlier work was performed with the organism known as cholera massowah, which is now known not to be a genuine cholera spirillum, nevertheless, his work has been confirmed for the latter organism by several investigators, among them Kolle and Wassermann. By others, however, this idea has not been accepted. The results of all of the author's experimental work on this subject have been in accord with the view that the toxin is an endotoxin. If 18-hour agar cultures of the cholera organism are suspended in sterile normal saline solution, filtered through a Reichel candle, and the filtrate injected into guinea-pigs in varying amounts, it will be observed that the filtrate possesses very little toxic power. On the other hand, if what remains on the filter is suspended and injected, even though the organisms are killed before injection, the animal dies with all the symptoms of cholera intoxication. Evidently the bacteria contain the toxin. If other agar cultures of the organism suspended in saline are carefully killed, for example, by heating for a brief period, and the bacteria are allowed to digest themselves by their own ferments for 2 or 3 days, ground in a mortar, and then filtered off, the filtrate obtained from these killed and digested organisms when injected into animals shows marked toxic properties. The filtrates of very young bouillon cultures of the cholera organism are also not toxic for animals, and only in filtrates of those cultures in which there are found numbers of dead bacteria which through autolysis have begun to disintegrate, is a toxic action observed. The filtrates of old bouillon cultures are much more toxic. Obviously all of this evidence is in favor of the view that the cholera toxin is an endotoxin, and experiments in immunization which have been made also support this view.

On the other hand, Metchnikoff, Roux, and Salimbeni believe that the living cholera organism produces a soluble diffusible toxin. A small, sterilized collodion sac, of a capacity of 3 or 4 c. c., was filled with peptone solution or nutrient bouillon, inoculated with the cholera spirillum, closed and placed in the abdominal cavity of a guinea-pig. Another guinea-pig received a similar sac containing a suspension of one and one-half gelatin cultures of cholera bacteria suspended in peptone solution and killed by chloroform, while a sac containing only peptone solution was placed in a third animal. The last animal remained unaffected. The one which received the suspension of dead bacteria showed a slight elevation of temperature and emaciation, while animals which had received the living organisms died in from three to five days with all the appearances of cholera intoxication. The collodion sac in these animals still showed motile spirilla. Attempts made by some other observers to repeat these experiments have not as yet been so successful.

Some objections, moreover, have been raised against these conclusions.
Many of the organisms in the collodion sac in the abdominal cavity of
an animal would after 24 hours die in large numbers, and through dis-
integration and plasmolysis the toxin would be set free from the bacterial
cells. The living organisms remaining would later give rise to additional
toxin in the same way, and thus the death of the animal would result.
In the case in which the dead organisms were inoculated in the sacs,
there was, as stated by the authors, an elevation of the temperature for
several days and emaciation occurred. The amount of cholera toxin pres-
ent was obviously not sufficient to bring about the death of the animal.
With the experiment performed with the living organisms, there would
be many successive generations from which additional amounts of toxin
would be furnished. Hence, the total amount of toxin set free would be
many times greater than that from the amount of killed organisms intro-
duced.

In order to produce this soluble toxin in artificial media, Metchnikoff,
Roux, and Salimbeni selected a highly virulent organism which was
grown in similar sacs in the abdominal cavity of a guinea-pig. In this
way a culture was obtained, 1/160 c. c. of which sufficed to kill guinea-
pigs. This organism was then grown in a culture medium consisting of
2 per cent. gelatin, 2 per cent. peptone, and 1 per cent. sodium chlorid,
with the addition of fresh guinea-pig serum from another sac. Cultures
from this medium after three or four days, when filtered, killed guinea-
pigs in from 16 to 24 hours, on being administered in amounts of 1/3 c. c.
per 100 gm. of body weight. The toxin thus obtained was not materially
changed on being boiled, but lost its toxicity on contact with the air and
on exposure to light. With such a toxin it was maintained that a highly
effective antitoxic serum could be produced in animals.

After three months' treatment of horses and goats with this toxin the
serum of the animal was effective in amounts of 3 c. c. against one and
one-half times the lethal dose. After six months 1 c. c. neutralized four
times the lethal dose.

Brau and Denier reported that they were able to obtain a very active
toxin from the cholera vibrio by growing this organism in a special culture
medium consisting of gelatin bouillon, normal serum of the horse, and
defibrinated blood, heated to 60° C. for 3 hours. Hemolysis occurred
after 24 hours. After 4 days' development the cultures had become lique-
sified. After 7 days they were filtered through paper and then through a
Chamberland F. candle. Certain precautions are necessary in order to
obtain the toxin in satisfactory amounts. They advised that the serum
be heated at 60° C. for 3 hours in order to destroy the substances antago-
nistic to the development of the cholera vibrio. The thermostat must be
kept at a constant temperature, variations even of 1° C. interfering with
the production of the toxin; the optimum temperature was found to be
between 38° and 39° C. It is also necessary for the cultures to be well
aèrated and shaken each day. Finally, the strain of cholera spirillum employed must not have been passed through animals, since such a passage diminishes the toxic power of the organism with great rapidity.

Following this method they were able to obtain a cholera toxin with 26 cultures of vibrios isolated in Saigon, with two strains obtained from the Pasteur Institute, one of which was isolated in Bombay and the other in Nasik, and with three strains from Egypt. They concluded that a soluble toxin may be obtained from vibrios isolated from cholera stools and that the production of the toxin may be increased by cultivating the organisms in their special culture media. In a later publication the same authors called attention to the fact that this cholera toxin manifested its effect quickly and without a period of incubation when injected into an animal. Guinea-pigs and rabbits could be immunized against the toxin so that they were able to resist two fatal doses injected at one time, and horses which had been inoculated intravenously at intervals of 6 months with 0.5 l. of the toxin, furnished a serum of which 0.02 c. c. neutralized two fatal doses of the cholera toxin after a contact of 30 minutes in vitro. The serum also exerted antimicrobial, agglutinating, and precipitating qualities. The cholera toxin was not destroyed by boiling and the boiled toxin produced as good serum as the unboiled one. It was also found that the injection of cultures of the living cholera vibrio into the veins of a horse furnished an antitoxic serum which was even more active than that prepared with the soluble toxin. They admit that the cholera toxin appears to be analogous to the endotoxins of the plague and typhoid bacilli, although in their final conclusions they state that the organism produces a soluble toxin the action of which is rapid and without a period of incubation. They also believe that the cholera toxin contained in the extracts of the bacteria and that obtained in the liquid culture media cannot be distinguished. The authors in their last article emphasize some further precautions to be observed in order to secure a good production of the toxin. The media finally employed consisted of 20 c. c. of normal serum of the horse plus 10 c. c. of defibrinated blood. The serum and defibrinated blood must be at least three weeks old before use, as otherwise almost no production of toxin occurs.

MacFadyan undertook experiments with sterile juices obtained from the cholera organism, the bacteria being ground at the temperature of liquid air, so as to preclude the possibility of chemical change, the organisms then being placed in ten times their weight of .1 per cent liquor potassae. Toxie extracts were obtained from the most virulent cultures which killed guinea-pigs acutely in doses of 0.1 to 0.5 c. c., while 0.02 c. c. rendered the animals ill. The endotoxin also exerted its action when injected subcutaneously in quantities of 1 and 2 c. c. Doses of 0.1 and 0.5 c. c. killed rabbits on intravenous injection. The juices deteriorated in toxic power on keeping, and the toxin was destroyed by heating at a
temperature of from 55° to 60° C. Goats were immunized with increasing doses of the endotoxin and a serum was obtained of which 0.002 c. c. neutralized from 3 to 4 ascertained lethal doses of the endotoxin for a guinea-pig. This property was not possessed by 1 c. c. of normal serum.

Kraus in working with a vibrio designated as "Nasik" was able to obtain a powerful toxin from filtered bouillon cultures of this organism. By heating to 50° C. its poisonous properties were destroyed. Kraus concluded that this organism was not a true cholera vibrio, owing to its agglutinative, bactericidal, precipitating, and hemolytic properties. Later he carried on experiments with a number of different vibrios and concluded that the cholera poison is a true soluble toxin, and may be destroyed by antitoxin.

Kraus prepared a serum for the treatment of cholera in man by the injection of the toxins from the El Tor vibrios, which, however, possess some variations from the cholera vibrio in their biological properties. He immunized goats or horses by the subcutaneous inoculation, either with bouillon filtrates or with agar cultures of these vibrios, or with extracts from the agar cultures. In the beginning he injected from 0.5 to 1 c. c. of the "toxin" and increased the amount at intervals of from 6 to 8 days until over 900 c. c. of toxin were injected into a horse during a period of ten months. With such a serum he succeeded after one hour in saving mice which had previously received the toxin or been infected. In guinea-pigs, if the injection of the serum was delayed for one-half hour after the injection of the toxin or of the infection, even large quantities of the antitoxin would not save the animal. Through the intravenous application of large doses of the serum guinea-pigs could occasionally be saved after one-half hour, but after one hour it was of no value. In 1903 the writer prepared an anti-endotoxic serum by the inoculation of an extract of the cholera organism made by killing the organisms carefully within a very brief period, digesting at 37° C., grinding, and submitting the suspension to a pressure of about 600 atmospheres, and, finally, filtering under pressure through a Reichel or Berkefeld candle. In this way sera were obtained of which 0.2 c. c. would neutralize 4 lethal doses of toxin when mixed immediately before inoculation. I also found, as MacFadyan has since done, that a temperature of 60° C. destroys most of this primary poison, or at least converts the toxin into toxoid.

It would appear that the toxin which Kraus has obtained from true cholera organisms, and which he designates as a secretion of the organism and as a soluble toxin, is none other than the one with which Braun and Denier, MacFadyan, and the writer worked, and that it should be rather regarded as an endotoxin, for convincing evidence to the contrary at least has not yet been brought forward.

Early attempts were made to obtain the immunizing substances from the cholera organism by the method of Lustig and Galeotti. The agar
cultures of the organism were dissolved in a 1 per cent. caustic potash solution and then treated with 1 per cent acetic acid. The resulting precipitate was filtered and washed to a neutral reaction. Horses were then immunized with this preparation for the purpose of securing a serum.

More recently Schurupoff extracted 1½ to 2 day cholera cultures with a weak alkali, the details of the method not being given, and obtained an endotoxin that, upon intraperitoneal injection, was very poisonous for guinea-pigs. Horses were inoculated intravenously with increasing doses of this toxin at intervals of 6, 7, and 10 days. In this manner he claimed that a pure anti-endotoxic serum was produced. However, this view has been opposed by Raskin and Horowitz, who showed that this serum had no favorable effect if it was given 4 hours after infection.

Carrière and Tomarkin have undertaken to produce a serum in horses and goats by the method employed previously by the author. They state that such a serum should possess besides agglutinating and bactericidal properties, also anti-endotoxic, complement-fixation, and opsonic properties. With this idea in view they have prepared a serum for use in man, inoculating the animals both subcutaneously and intravenously with living and killed cholera organisms, as well as with the intracellular substances obtained from the bacteria by shaking them in salt solution.

Pottevin, recently working with three cholera vibrios, found differences in toxin production when the organisms were grown in a special bouillon. Two of the organisms produced a toxin which was rapidly fatal to young rabbits and pigeons when injected intravenously; and was strongly hemolytic. Both of these substances were thermolabile, one hour at 50° C. destroying the hemolysin totally, and the toxin in great part. The toxic effect left was not removed even by prolonged heating at 100° C. The thermostable fraction was only one-quarter to one-eighth of the toxic power of the original and did not affect pigeons at all. The third vibrio produced a toxin similar to that described by Roux, Metchnikoff, and Salimbeni. Intravenously in rabbits it caused death. Immunization carried out in animals produced an antitoxin to the thermolabile poison, but no antibody to the thermostable portion.

Horowitz (1913) has very recently studied anew the question of the cholera toxin and has found that cultures of the organism made in 1 per cent. glucose bouillon seem most favorable for the production of the toxin. The filtrate of such cultures was found to be most toxic, and it was found that the toxicity was not proportional to the life of the culture, but rather to the number of dead bacteria present in it. The glucose bouillon cultures appeared to be sterile on the third day. Marked acidity developed in the cultures and it was thought that the acid produced served as a much more delicate extracting agent than those which have been commonly employed. Extracts of the organism were obtained which were fatal to guinea-pigs in ½ c. c. doses. The toxicity of the solution
was apparently due to endotoxin. Two animals immunized with the filtrates showed that a serum could be produced which would remove the toxicity of the bouillon filtrates. Such sera, however, had very low agglutinating and bacteriolytic powers. Whole glucose bouillon cultures of three or four days' growth produced sera which had high agglutinating and bacteriolytic values, and also had the power to a certain extent of destroying the toxicity of filtrates. Sera, on the other hand, made by immunizing rabbits with heated agar cultures had high agglutinating and bacteriolytic values, but did not destroy to any great extent the toxicity of filtrates. Horowitz believes that the toxin-destroying body of these sera is not the same as the bacteriolsyn, but is related to it as a peptolytic is to a proteolytic ferment. In the experimental infections of animals the serum made by the injection of heated cholera organisms was not so efficacious as a serum made by the injection of three- or four-day-old bouillon cultures. The destruction of the toxin by any of the sera seemed to be a complement action.

Finally, Rotky during the present year has attempted to obtain an anti-aggressin serum against cholera. This author found that, by the treatment of mice with cholera aggressin, they could be immunized against subcutaneous as well as intraperitoneal infection of multiple lethal doses of the cholera organism. An immune serum of a guinea-pig obtained by several injections of sterile aggressin, after it has been robbed of its bactericidal immune properties by treatment with large quantities of killed vibrios, protected guinea-pigs and mice against a multiple infection of the El Tor vibrios. It should be noted that the experiments were made with the El Tor vibrios which, as has already been noted in this article, have exhibited some peculiarities in regard to toxin production, frequently, at least, not shown by other cholera vibrios.

Moreover, the previous work of Wassermann and the author must be considered in connection with these experiments. By this work it was shown that the immunity obtained by the inoculation of aggressin exudates was of no different quality as regards specific immunity from that obtained by the inoculation of living cultures of the organism.

Very recently Zinsser and Dwyer have performed experiments which seem to suggest that the aggressins of Bail may be of the nature of anaphylatoxin, and that the invasive properties of bacteria may well be greatly enhanced in the animal body, as contact with the serum induces the formation of these substances. The experiments suggest that the action is not specific in this respect.

The Immunizing Properties of Cholera Sera.—When cholera immune serum is prepared by repeated inoculations of an animal with killed or living agar cultures of the cholera organism, the properties which such a serum exerts in its protection of a susceptible animal are mainly bactericidal. If a guinea-pig is inoculated intraperitoneally with 1 loop of a
virulent cholera culture (of which the lethal dose is \( \frac{1}{1000} \)), and at the same time or a little later the animal is inoculated in the same manner with a cholera immune serum obtained as indicated above, the cholera organisms are quickly broken up and destroyed and the animal survives the infection. If, however, the inoculation of the serum is delayed for one or two hours after the time of the infection with the living vibrio, then, even though very large doses of the serum are given, the animal dies of intoxication. In this instance, although the great majority of the vibrios are disintegrated and destroyed by the serum, the organisms have increased so rapidly in numbers that when they are destroyed sufficient endotoxin is elaborated from the bacterial bodies, together with that which results from the few surviving organisms, to cause the death of the animal later. If the injection of the serum is delayed until several hours after the inoculation with the living organism, that is, until a time when the animal is beginning to suffer from intoxication, then, even though very large amounts of the serum are injected, practically very little destruction of the bacteria occurs, owing largely to the lack of suitable complement in the serum of the guinea-pig. In spite of this failure, however, nothing will save the animal, not even the addition of fresh complement, since there is already at the time sufficient endotoxin present in the vibrios to cause the death of the animal, and the serum possesses no antitoxic properties in sufficient amount to neutralize the effect of the endotoxin. Moreover, if one first kills, for example with chloroform, the same virulent cholera organism, and inoculates the guinea-pig intraperitoneally with the lethal dose of the killed organism (about 4 or 5 loops), simultaneously with the immune serum, although a union occurs between the bacterial amboceptors of the serum and the corresponding receptors of the vibrios (a fact demonstrated by other experiments), nevertheless the animal dies for the same reason expressed before, namely, that a lethal dose of cholera endotoxin in the bodies of the dead organisms becomes liberated by their disintegration, without there being sufficient antitoxin in the serum to neutralize the action of this endotoxin.

If such difficulties then are encountered in attempting to save guinea-pigs from cholera infection by such cholera immune sera, it might be accepted a priori that but little benefit would be obtained from their use in the treatment of cholera in man, even though the symptoms of cholera infection are so unlike in these animals and man.\(^1\)

In man also the small intestine offers a more favorable location for the development of the cholera vibrios, and one where the serum cannot come into actual contact with the developing organisms and exert its bactericidal properties to the same extent as it can in the abdominal cavity of the guinea-pig. Moreover, even the fact that the serum in man may be given

\(^1\) Attempts at cholera infection of young rabbits or monkeys by the mouth have not produced sufficiently definite results to be of any value in the consideration of this question.
in greater quantities and is excreted in larger amounts from the intestine will not give it the same advantage of action in this respect as it would have in the abdominal cavity of the guinea-pig, and, in fact, it has been shown that, in cases of cholera with symptoms of marked intoxication, the use of these bactericidal sera has not produced any apparent beneficial effect.

**Effect of Serum in Man.**—On the other hand, the injections of large amounts of the different cholera immune sera has apparently exerted no injurious action, either temporary or permanent; upon the patients so treated with them, and even in those cases in which the functions of the kidneys have been suspended no injurious effects have been observed from the administration of the serum.

The opinion earlier expressed that the bactericidal effect which the serum would exert in the intestine after intravenous injection might lead to more acute intoxication through the rapid destruction of the spirilla does not seem to be justified from the observations which have been made in relation to the treatment by serum of the disease in man.

Owing to the lack of success from the employment of bactericidal sera in the treatment of cholera, the trend of scientific investigation in relation to the serum treatment of the disease has been in the direction of the preparation of the antitoxic sera, which we have already considered, and the results of treatment in man with these sera will now be discussed.

**Treatment in Man.**—Brau and Denier, whose investigations have already been considered, prepared two sera for the treatment of cholera in man. Serum A was prepared by injecting a horse with the cholera toxin entirely free from bacteria, and the second one, serum B, by injecting a horse with the living organisms and toxin. These sera were examined by the author, and were found to possess specific agglutinative and bactericidal properties, one showing a much higher value in this respect than the other. No study was made of the neutralizing power of the sera for lethal amounts of the filtered cholera toxin. Guinea-pigs inoculated with 1 c. c. of serum B and at the same time with 1 or even 2 loops of a cholera vibrio, of which the lethal dose was .1 loop, survived the inoculation; however, when they were inoculated with 5 loops and 2 c. c. of the serum, they invariably succumbed. Pfeiffer's phenomenon seemed to be complete, as was shown by the post-mortem examination of a number of these animals, since microscopic preparations from the exudate in the abdominal cavity showed no motile vibrios and the animals had apparently died rather from an intoxication than from an infection. However, these experiments obviously do not demonstrate whether death had occurred from the effect of the endotoxin contained in such a large amount of the spirilla (5 loops) or from the effects of another soluble toxin.

Serum B was found to protect against larger doses of the living organ-
ism than serum A, as was proved by testing the bactericidal power of the two sera. The bactericidal value of the sera was apparently, at all events so far as the living organisms were concerned, the most important factor in protecting the animals, at least up to a certain dose. In many of the animals which died and which had not received excessively large doses of the cholera spirillum Pfeiffer's phenomenon was also found to be complete, or almost so.

In all, 52 human cases of cholera were treated by Dr. Denier with the sera. In each instance a careful bacteriologic diagnosis of cholera was made both by Dr. Denier and by the writer. The injections of the sera were given intravenously and in large quantities, as much as 250 c. c. in a liter of Hayem's solution being inoculated at a single dose. Following this primary inoculation 100 c. c. of serum was injected in an equal amount of saline solution every 3 hours until a reaction on the part of the patient occurred. The average amount of serum given was from 300 to 500 c. c., but in one case 1,000 c. c. was injected in 24 hours. The cases in the hospital were treated alternately with serum, that is, every other case admitted received this treatment. The injections of the serum were usually given very shortly after the time of the admission of the case to the hospital. Obviously, the patients were frequently in collapse at the time of their arrival. The following table shows the results of the serum treatment:

<table>
<thead>
<tr>
<th></th>
<th>Number of Cases</th>
<th>Cholera Spirillum Not Isolated from the Stools</th>
<th>Dead</th>
<th>Recovered</th>
<th>Percentage of Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>21</td>
<td>3</td>
<td>13</td>
<td>5</td>
<td>72</td>
</tr>
<tr>
<td>Serum A, antitoxic</td>
<td>16</td>
<td>1</td>
<td>11</td>
<td>4</td>
<td>75</td>
</tr>
<tr>
<td>Serum B, antimicrobial</td>
<td>5</td>
<td></td>
<td>2</td>
<td>3</td>
<td>40</td>
</tr>
</tbody>
</table>

From this table it is evident that the cases which received the antitoxic serum were not benefited by it, the mortality being even higher than in the ones which received no serum. The number of cases which received the antimicrobial serum is too small to justify decided conclusions, although the mortality is much lower.

The effect of treatment with other of these sera prepared with the idea of possessing antitoxic properties has been particularly observed in the epidemic of cholera in Russia in 1908-1909. Berthenson of St. Petersburg has reported upon 636 individuals who were treated with various cholera immune sera. Those employed were the sera of Kraus, Salimbeni, Schurupoff, and of Kolle, Carrière, and Tomarkin. Of the cases treated with serum 322 died, or a mortality of 51.2 per cent. Since about one-half of those attacked with cholera usually recover with various
methods of treatment, the results offer no indication of any value for the serum treatment employed as a whole. Other reports show that 133 cases were treated with the serum of Kraus and of Salimbeni in several different hospitals, and the favorable effect of the serum as employed in these institutions appeared doubtful, according to the reports of Kernig, Ketischer, and Jegunoff. Other investigators, however, believe the serum to have been of value.

Berdnikoff employed the Schurupoff serum in 49 cases in doses of from 40 to 50 c. c. diluted 2 to 3 times its volume with physiological salt solution. The injections were usually given intravenously. Only in one group of ten cases was a distinct favorable action obtained, the mortality being 36 per cent. against the general mortality of 70 per cent. In the remainder of the cases treated with the serum no favorable effect was noticed.

Stihlern, however, has reported more favorable results with Schurupoff's serum, particularly when larger doses were used. In the algid stage repeated intravenous injections of the serum were given with a large amount of sodium chloride solution. The saline injections were also given in intervals between the serum injections, and during the typhoid stage intravenous and subcutaneous injections were combined. In a later communication he summarizes his results in the following table:

<table>
<thead>
<tr>
<th>Quantity of Cholera Serum Injected, in Cubic Centimeters</th>
<th>Number of Treated Cases</th>
<th>Recovered</th>
<th>Died</th>
<th>Mortality Per Cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>60 — 90 (300)</td>
<td>25</td>
<td>14</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>200 — 400 (300)</td>
<td>79</td>
<td>56</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>400 — 600 (500)</td>
<td>27</td>
<td>27</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>600 — 800 (700)</td>
<td>26</td>
<td>18</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>800 — 1000</td>
<td>19</td>
<td>10</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>1040 — 1390</td>
<td>11</td>
<td>6</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>187</td>
<td>131</td>
<td>56</td>
<td>29.9</td>
</tr>
</tbody>
</table>

The maximum quantity of serum that was injected intravenously within 12 hours amounted to 600 c. c. In the most severe cases as much as 800 c. c. was injected in 36 hours. The cases which were complicated with uremic coma received also subcutaneous injections of the serum, 60 c. c. per day in a course of 5 to 7 days. Some of the most severe cases received as much as 18 l. of saline solution. One hundred and forty-nine of the 187 cases underwent a very severe attack of cholera with a marked algid stage. Of these 93 recovered and 56 died, a mortality of 37.5 per cent. Twenty-five cases were moderately severe and showed a distinct algid stage; all recovered. In 13 mild cases in which serum was given, all also recovered. In 228 cases which received sodium chloride solution intravenously and no
serum the mortality was 42 per cent., and of 142 cases that were treated with subcutaneous injections of salt solution the mortality was 54.9 per cent.

In connection with these statistics, however, it must be recalled that Rogers by his method of treatment with intravenous injections of hypertonic saline solution and of the administration of permanganate of potash claims a mortality of but 23.3 per cent. without serum, and several other observers—Logan, Whyte, Bishop, Megaw, and others—employing his method without serum, report almost as good or better results as regards general mortality than those cited by Stühler.

In a further communication Stühler reports upon his series of cases treated partly with serum plus physiological salt solution and partly with physiological salt solution alone. Of 742 cases that received neither serum nor systematic intravenous injections of salt solution 407, or 54.9 per cent., died. Of 193 patients who received systematic saline injections but no serum 64 died, or 33.2 per cent. Of 153 patients who received infusions and also serum 46 died, or 30 per cent. He believes that if the cholera serum is prepared in a proper manner it possesses a certain therapeutic effect.

Salimbeni has reported upon 42 cases treated with his serum at St. Petersburg with a mortality of 23.8 per cent., while the general case mortality in the official returns was 45.6 per cent. The serum was injected subcutaneously, as a rule in doses of 100 c. c. in 400 to 500 c. c. of saline solution often repeated. The intravenous injections were given in cases in which the conditions for resorption were not favorable. The author reports that the beneficial results were apparent in the improvement of the pulse and the disappearance of the cramps. In this connection, however, it must be mentioned that such symptoms usually disappear also after the injection of saline solution alone. Seven of the cases which he treated were of moderate severity and six were light cases. None died. Of 10 severe cases, 1 died; while of 19 very difficult cases 9 died, a mortality of 47.3 per cent., as compared with a mortality of 75 per cent. among such cases which received other treatment.

Stühler has reported upon the treatment of 94 cases in three hospitals in St. Petersburg which were treated with Salimbeni's serum, of which 59 died, a mortality of 62 per cent. Other observers also thought the serum was of little value.

In regard to the serum produced by Kraus, reports have been made by Jegunoff. He used doses up to 140 c. c. with 500 to 700 c. c. of saline solution injected intravenously. Twelve patients were treated in this way with a mortality of 25 per cent., as compared with a general mortality of 75 per cent. in cases which received no serum. In the cases in which no improvement resulted after the first injection, the second injection of from 80 to 120 c. c. seemed of no benefit. In cases in which the
patient after the first injection escaped the algid stage, but which later showed anuria for 2 or 3 days, also the repeated injection of 80 to 120 c. c. did not prevent the development of paroxymal nephritis, nor a fatal result. The number of cases treated is too small to draw any conclusions.

Hundögger treated 35 cases with Kraus' serum in doses of 100 c. c. mixed with 2 l. of sodium chlorid solution and injected intravenously. In some cases 100 c. c. was given intravenously, 50 subcutaneously, and 50 by mouth, in all about 200 c. c. The mortality was not reduced by the serum. Moreover, it appeared to exercise no influence upon the course of the disease and did not prevent the development of uremia.

A number of other observers have also failed to see any favorable action of the serum of Kraus upon the course of the disease or upon the mortality. Albanus treated 54 cases in which the mortality was 57.5 per cent., as compared with a mortality of 84.3 per cent. in untreated cases. Kraus himself has assumed a therapeutic value for his serum upon the basis of observations upon 119 cases that were treated by Ketscher and Kernig. Of the 70 cases treated subcutaneously about 58 per cent. died; of the 35 cases treated intravenously by Kernig, 51.3 per cent. died; of the 12 cases treated by Ketscher, 50 per cent. died, as compared to a general mortality of the severe untreated cases of 69.4 and 50 per cent. Kraus recommends the intravenous injection of serum at the earliest possible time in doses of 60 c. c. with 100 c. c. of physiological salt solution.

In his recent report of the cholera epidemic in Bulgaria in 1913 the statistics of serum treatment are not given, but the value of treatment with hypertonic solutions of saline solution is referred to.

With the serum prepared under Kollé's direction by Carrière and Tomarkin 7 cases have been treated, 3 very severe, 2 severe, and 2 moderately severe. Only 1 of the very severe cases died. The entire quantities of serum for the different cases varied between 80 and 120 c. c. Besides the serum there were also injected large quantities of sodium chlorid solution intravenously. An unfavorable effect of the serum or appearances of serum disease were not observed in any of the cases.

**Indications for Other Methods of Treatment.**—In connection with the serum treatment of cholera, it is important to consider the factors which give rise to the special symptoms of the disease. In the stage of evacuation frequently within a few hours several quarts of fluid containing salts may be passed from the intestine or from the stomach by vomiting. This brings about an extreme dehydration of the tissues and blood, a fall in blood pressure and surface temperature, marked exhilaration or loss of the pulse, shrinking of the skin, muscular cramps, and suppression of urine. The local effect of the spirilla in the intestinal mucosa which is manifested by severe catarrh may be sufficient to explain some of the in-
testinal symptoms, such as the copious exudations, the violent diarrhea, and perhaps vomiting; but the heart failure, cyanosis, and nephritis, and other accompanying symptoms, which also result cannot be explained in this manner. These differences may be brought about, first, by the enormous abstraction of water and salts both from the blood and from the tissues, and, secondly, by the action of toxic substances produced by the cholera spirillum and absorbed from the intestine. It is not necessary to refer to the quantitative and qualitative changes in the blood in relation to this discussion. Just how much the anuria and the nephritis occur as a result of the abstraction of the water and salts from the blood and tissues, and just how much they are due to the action of the cholera toxin, is not at the present time altogether clear. However, it seems unquestionable that the abstraction of such enormous amounts of water and salts from the tissues, resulting as it does in the increased concentration of the blood, its loss in volume and salts, and consequent rapid fall of blood pressure, must play a very important rôle in the production of collapse, and consequently in the interruption of the blood supply of the kidney with the resulting damage to its parenchymatous cells.

Hence, any successful treatment for cholera must combine methods not only to neutralize or eliminate the action of the toxin, but to restore the lost water and salts, and to improve the condition of the circulation and restore the blood pressure. Practically all observers recognize the value of intravenous injections of saline solution in the treatment of Asiatic cholera. This method of treatment was employed with success by the author in the cholera epidemic in the Philippines in 1902, and in several subsequent epidemics.

In the treatment of this disease during epidemics in the past year a number of observers—Bishop, Megaw, Lang, Defressine, Oudard, Van Dijken, Roelfsena, Whyte, Gallas, Newton, and Logan—have relied particularly upon this method of treatment rather than upon serum treatment.

The treatment of the disease by the injection of saline solution according to the method advocated by Rogers, Sellards, and others, has already been considered by Wherry elsewhere in this work and will not be referred to here.

While as yet no satisfactory serum for the treatment of Asiatic cholera has been prepared, nevertheless the recent experimental work carried on in reference to this subject is not discouraging to the idea that a satisfactory antitoxic serum may eventually be prepared for this disease.

**Treatment by Enemata of Serum**

Salimbeni and Orticoni (1913) have recommended treatment with serum employed as enemata for the purpose of ridding the intestine of cholera carriers, or those with very mild symptoms of the disease of the
cholera spirillum. The cases first received an evacuating enema, and immediately after this an enema of 50 c. c. of cholera serum in 200 c. c. of salt solution, the fluid being slowly injected, and an attempt being made to let the injection penetrate as far into the intestine as possible. Thirty-four cases in which cholera vibrios were found in the feces, some of which had slight choleraic diarrhea, were treated in this manner. In 9 out of 34 the vibrios had disappeared at the time of the first injection; in 22 of the remainder they disappeared within 2 days; and in 3 others in from 3 to 6 days after the injection. None of the cases developed more marked symptoms of cholera. In a number of other cases observed as controls, several carriers continued to excrete the cholera organism for 15 days, and at least 2 developed cholera and died. The authors therefore consider that this method of treatment is worthy of a further trial in this class of patients.

REFERENCES

—. Ibid., 1906, cxxii, 728.
Defressine. Arch. méd. et pharm. nav., 1912, xcviii, No. 8, 104; No. 9, 194.
Jegunoff and Ketscher. Russky Wratsch., 1909, No. 11.
Kernig. Russky Wratsch., 1909, No. 36.
—. Ibid., 1906, xli, 15.
—. Ibid., 1907, No. 42.
—. Ibid., 1908, No. 26.
—. Ibid., 1908, xlv.
—. Handb. der Technik u. Methodik der Immun., 1908, i.
—. Ibid., 1909, Bd. ii.
REFERENCES

Megaw. Lancet, 1912, 1424.
Newton. Medical Missions in India, 1913, xix, 143.
——. Ibid., 1910, T. xxiv.
——. Russky Wratsch, 1909, Nos. 18 and 19.
——. Appendix to a History of Asiatic Cholera in the Philippine Islands, Manila, Bur. of Printing, 1909.
——. Russky Wratsch, 1909, No. 1.
——. Ibid., Nos. 17 and 18.
CHAPTER XI

WHOOPING-COUGH (PERTUSSIS)

E. MATHER SILL

To Bordet and Gengou (2) we are indebted for a full description of the bacillus of whooping-cough. They discovered the bacillus in 1900, and in 1906 obtained it in pure culture. This organism is described as a small Gram-negative cocco-bacillus, closely resembling in size and shape the bacillus of influenza, but, examined in the original cultures, it is usually rather longer and plumper.

The most recent knowledge of the organism of whooping-cough has come to us through the work of Mallory and Horner (8), who had the opportunity of examining the tracheas and lungs of three patients that died of uncomplicated whooping-cough in the Boston City Hospital. These observers found large numbers of minute bacteria between the cilia of many of the cells lining the trachea, ovoid in form, and Gram-negative, which resembled closely the bacillus discovered by Bordet. In most instances the micro-organisms lay at the base of the cilia, with the long axes extending in the direction of the cilia. In the secretions of the trachea and bronchi were found masses of bacteria similar to those found between the cilia.

Mallory successfully inoculated rabbits and puppies by placing a few drops of a veal bouillon culture in the nares. The chief symptoms in the puppies were coughing and sneezing, which, after a few days, became more and more paroxysmal, with some secretion from the nose and eyes. The chief symptom in the rabbits was emaciation. Several puppies kept in the cage with one that was inoculated acquired the infection either by direct contact or contagion. After varying periods these animals were killed and showed lesions in every way corresponding to those found in man, but the number of organisms was less.

A rabbit was inoculated with a strain originally obtained from Bordet. After eight days it was killed and the bacillus was reobtained in pure culture in the nares, trachea, and bronchi. Thus Mallory demonstrated the causal relation of the Bordet-Gengou bacillus to whooping-cough. There seems to be no question now as to the scientific and practical basis.
for the use of this bacillus in the form of a vaccine for the treatment of whooping-cough.

DIAGNOSIS

In a typical case of whooping-cough, especially after the convulsive stage has begun, the diagnosis is easy. There are a number of conditions, however, that may give symptoms closely resembling this disease. A spasmodic cough, due either to simple or tuberculous enlargement of the bronchial glands, may suggest pertussis. Hypertrophied tonsils some-

![Image](https://via.placeholder.com/150)

**Fig. 1.—Bordet's Pertussis Bacillus Pure Culture. Carbol-fuchsin. Magnified 1,200 diameters.**

...times produce a cough. There is occasionally seen a hysterical imitation of the cough of pertussis. Cases that present great difficulties of diagnosis are those with very mild attacks. Such children may go through the whole course of the disease without once having a typical paroxysm, and never have a whoop, or a child may have perhaps one or two whoops during the whole course of the disease. Abortive cases may be very difficult of diagnosis. In early infancy nearly every cough is more or less spasmodic in character, and in some cases of bronchitis, where there is also laryngitis, one may hear a whoop which quite closely resembles that of pertussis; on the other hand, these young infants, when suffering from whooping-cough, rarely exhibit a long-drawn inspiration with the resulting crowing sound or whoop following a paroxysm of coughing. It is in these conditions simulating pertussis, and in atypical forms of the disease, that the serological reactions may come to our aid.
Improvement of symptoms under vaccine may also be of some value in later diagnosis of suspicious cases.

**Seroserology.**—It has been shown that there is an antibody formed in the blood in whooping-cough which agglutinates the Bordet-Gengou bacillus. By using this, test cases of whooping-cough may be diagnosed when symptoms are indefinite. Povitsky (11) in a study of the agglutination in 59 pertussis cases has shown that a strongly agglutinating serum is best obtained in the rabbit by 10 or 12 intraperitoneal inoculations of living culture given at several day intervals. Over 95 per cent. inoculated in this manner produced agglutinins. A high-titer serum (1:4000 to 1:10,000) was always a quickly acting serum, while a low-titer serum was slow in reaction. Bacillus pertussis strains can be specifically identified by agglutination tests from hemoglobinophilic bacilli, pertussis-like bacilli, and Bacillus bronchisepticus. Cross agglutination between these organisms did not occur higher than 1:40. A strong serum agglutinates all pertussis strains equally well, but not equally promptly in the highest dilutions (1:2000-2500). For a positive diagnosis by the agglutination test a dilution of 1:200 is necessary, since out of 51 controls about 33 per cent. of nonpertussis adult sera showed agglutination in up to 1:40; 17 per cent. in up to 1:100. Forty per cent. control children's sera (scarlet fever cases) agglutinated in up to 1:40. This test compares favorably with the complement fixation test only in the first week of the whoop. In later stages complement fixation antibodies are more frequent than agglutinins. Olmstead and Luttinger (9) made complement fixation tests on serum from 111 cases of pertussis and suspected pertussis, and about 40 per cent. gave a positive reaction with antigens of the Bordet-Gengou (2) bacillus when an active serum was used. Convalescent vaccination cases have shown the highest per cent. of positives. Bordet and others have obtained reactions of complement-fixation using the specific bacillus as antigen, and this reaction gives promise of considerable aid in the diagnosis of obscure cases. The limitations of the reaction of both agglutination and complement-fixation have not been entirely determined, but from the work already done it would appear that strong positive reactions with suitable controls have a positive diagnostic value.

**VACCINE TREATMENT**

The most recent and, to my mind, the most effective and rational method of treating whooping-cough is by means of a vaccine made from a culture of the Bordet-Gengou bacillus. I have used this method of treatment almost exclusively for several years, and in all have treated sixty-one patients, with better results than I had previously obtained by means of drugs. In the early patients treated 20,000,000 of the killed bacteria were given every three to seven days. The results with this dose seemed better
1. Ciliated epithelium lining normal trachea of child. × 1,000.

2. Ciliated epithelium lining trachea of child dying in acute stage of whooping-cough. Large numbers of bacilli are present between the cilia. × 1,000.

3. Ciliated epithelium lining bronchus of child; mucus forming in cells and collecting on surface. × 1,000.


5. Minute bacilli present between cilia of two cells lining trachea. × 1,500.

6. Desquamating epithelial cell in trachea with numerous bacilli between cilia. × 1,500.


Fig. 2.—Epithelial Cells of Trachea and Bronchus of a Child under Normal Conditions and in Whooping-Cough. (After Mallory and Horner.)
than those obtained by drugs, but later it seemed advisable to increase the size of the inoculation, and a 40,000,000 dose at intervals of two or three days was given. This increase in dosage with a shorter average interval between inoculations was decidedly beneficial, shortening the attack on the average by at least a week. In very severe cases with many paroxysms, where 40,000,000 at a dose did not seem to control the paroxysms, or bring relief, 60,000,000 were given with gratifying results. This was my first series of 33 patients, in all of which the effect of the vaccine was to diminish the severity and the number of paroxysms and amount of vomiting. I was guided largely by the severity and number of paroxysms in giving the vaccine. Children with severe attacks received larger doses at more frequent intervals than those with a milder affection and fewer paroxysms. The youngest child treated was one month of age, and the oldest ten years; the majority were from six months to three years.

Patients seen early in the disease, before the paroxysms had attained their height, responded quickly, and their course was shorter. The number and severity of the paroxysms and the vomiting, when present, in all cases, after from one to three injections, was decidedly lessened.

The younger children usually did better than those older, which was probably due to the fact that they got proportionally larger doses.

It was found that the children did better when moderately large doses of from 40- to 60,000,000 bacteria were given at frequent intervals of two days. (Cases 20, 30, 31, 37, 38, 39, 40, 41, 42, 43, 44, 45, 47, 48, 51, 52, 53, 55, 56, in table on pages 302 and 303, illustrate this fact.)

In the first series of 33 patients the average length of time required to effect a cure when all cough had disappeared was 4½ weeks; in the next series of 13 where larger doses at more frequent intervals, on an average, were given the average length of time to bring about a cure was 3½ weeks, while in the last series of 13 where a still larger average dose was given the time elapsing until recovery was further shortened in a number of instances from one to three weeks, while the whole duration of the disease varied, as will be seen by the appended table, from two to nine weeks. Further experience with the use of pertussis vaccine has shown that much larger doses can be given with perfect safety, and with such rapidly ascending doses a quick amelioration of symptoms may be confidently expected. My experience leads me to believe that to obtain the best results the pertussis vaccine made from the original strain of bacillus obtained from Bordet should be given early in the attack in doses of 100,000,000 to 200,000,000 and doubled every two to three days, according to the age of the child and severity of the disease. In giving the vaccine it is advisable to start, in babies and young children, with a fairly small initial dose, which may be increased as symptoms may indicate. In a few young babies, where very large doses of the vaccine had been given, I have seen what seemed to me to be a negative phase of short duration ovi-
VACCINE TREATMENT

enced by an increase in the number of paroxysms and an exaggeration of other symptoms. This negative phase was of only short duration.

None of the children had any very serious complications, except one who contracted pneumonia, and ran a typical course with high fever and consolidation of the lung; this was a child thirteen months old (case 51), who had been coughing for six weeks, and had over 40 paroxysms in twenty-four hours, when it came under treatment. A number of other patients suffered from bronchitis, sublingual ulcer, and subconjunctival hemorrhages, but as a rule the complications were few and not of a serious nature.

The vaccine is given under antiseptic precautions subcutaneously by first applying a little tincture of iodin over the site of the injection, which is usually the abdomen, buttocks, or arm. An ordinary glass hypodermic syringe is used. There is no constitutional or even slight local reaction following the injection of the vaccine. All the cases I have treated by this means have made complete recoveries. Commercial vaccine prepared from the original Bordet cultures was used in all my early cases. The first series of cases were given a vaccine containing Bordet's bacillus alone, while the second series received a combined vaccine containing

<table>
<thead>
<tr>
<th>Bacillus pertussis</th>
<th>50,000,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>20,000,000</td>
</tr>
<tr>
<td>Micrococcus catarrhalis</td>
<td>20,000,000</td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>10,000,000</td>
</tr>
</tbody>
</table>

Luttinger (7), who has treated a large number of cases with pertussis vaccine in the Department of Health whooping cough clinics in New York, has been using much larger doses with apparently better results and no untoward effects. (Rarely more than three injections were given.) His initial dose is 250 million bacteria, which is doubled every other day, provided there is no reaction. That is, the second dose is 500 million, the third one billion, and the fourth two billion. In nearly 3000 injections given he has never seen a severe reaction as evidenced by temperature of 103° to 100° F., accompanied by malaise or vomiting, diarrhea and chills. My more recent experience in giving approximately the same large doses of the specific vaccine as put up by the New York Bureau of Laboratories would seem to corroborate his observations. Of 138 cases which were treated by Luttinger with vaccines, 115 were seen during the first three weeks of the paroxysmal stage, and their average duration was 25 days; while the average duration of 35 similar cases treated with drugs (antipyrin and sodium bromid) was 40 days.

The effect of the vaccine treatment on a case of pertussis is first a decrease in the severity of night paroxysms, then vomiting usually stops, and the day paroxysms become less severe. This is followed after a few days by
## Synopsis of Cases Treated with Pertussis Vaccine

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age</th>
<th>Month of Year Sick</th>
<th>Duration Before Vaccine</th>
<th>Cough Day</th>
<th>Night</th>
<th>Vomiting Day</th>
<th>Night</th>
<th>Vaccine No.</th>
<th>Size</th>
<th>Dose, Interval</th>
<th>Vomiting Ceased</th>
<th>Duration Disease After Vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4 wks.</td>
<td>June</td>
<td>1 wk. 5 days</td>
<td>24</td>
<td>23</td>
<td>Yes</td>
<td>Yes</td>
<td>10</td>
<td>20 Mil.</td>
<td>3 days</td>
<td>4 days</td>
<td>4 wks. 1 day</td>
</tr>
<tr>
<td>2</td>
<td>3 yrs.</td>
<td>June</td>
<td>4 wks.</td>
<td>20</td>
<td>20</td>
<td>Yes</td>
<td>Yes</td>
<td>7</td>
<td>20 Mil.</td>
<td>4 days</td>
<td>8 days</td>
<td>4 wks. 3 days</td>
</tr>
<tr>
<td>3</td>
<td>7 mo.</td>
<td>June</td>
<td>5 wks. 3 days</td>
<td>30</td>
<td>30</td>
<td>Yes</td>
<td>Yes</td>
<td>10</td>
<td>20 Mil.</td>
<td>4 days</td>
<td>25 days</td>
<td>5 wks. 1 day</td>
</tr>
<tr>
<td>4</td>
<td>8 mo.</td>
<td>June</td>
<td>3 days</td>
<td>10</td>
<td>7</td>
<td>No</td>
<td>No</td>
<td>7</td>
<td>20 Mil.</td>
<td>3 days</td>
<td>5 wks.</td>
<td>5 wks.</td>
</tr>
<tr>
<td>5</td>
<td>5 yrs.</td>
<td>June</td>
<td></td>
<td>20</td>
<td>12</td>
<td>No</td>
<td>No</td>
<td>4</td>
<td>20 Mil.</td>
<td>4 days</td>
<td>5 wks.</td>
<td>5 wks.</td>
</tr>
<tr>
<td>6</td>
<td>3 yrs.</td>
<td>June</td>
<td>3 wks.</td>
<td>8</td>
<td>4</td>
<td>No</td>
<td>No</td>
<td>5</td>
<td>20 Mil.</td>
<td>5 days</td>
<td>9 wks.</td>
<td>5 wks. 1 day</td>
</tr>
<tr>
<td>7</td>
<td>4 yrs.</td>
<td>Mch., Apr., May</td>
<td>3 wks. 3 days</td>
<td>6</td>
<td>10</td>
<td>No</td>
<td>No</td>
<td>8</td>
<td>20 Mil.</td>
<td>7 days</td>
<td>42 days</td>
<td>7 wks. 3 days</td>
</tr>
<tr>
<td>8</td>
<td>4 yrs.</td>
<td>Sept., Oct.</td>
<td>2 wks. 3 days</td>
<td>12</td>
<td>12</td>
<td>Yes</td>
<td>Yes</td>
<td>6</td>
<td>40 Mil.</td>
<td>3 days</td>
<td>18 days</td>
<td>4 wks. 1 day</td>
</tr>
<tr>
<td>9</td>
<td>1 yr.</td>
<td>Aug., Sept., Oct.</td>
<td>3 wks.</td>
<td>8</td>
<td>5</td>
<td>Yes</td>
<td>Yes</td>
<td>12</td>
<td>20 Mil.</td>
<td>7 days</td>
<td>42 days</td>
<td>7 wks. 3 days</td>
</tr>
<tr>
<td>10</td>
<td>3 yrs.</td>
<td>Sept., Oct.</td>
<td>2 wks.</td>
<td>8</td>
<td>16</td>
<td>Yes</td>
<td>Yes</td>
<td>6</td>
<td>40 Mil.</td>
<td>4 days</td>
<td>26 days</td>
<td>3 wks. 3 days</td>
</tr>
<tr>
<td>11</td>
<td>2 yrs.</td>
<td>Aug., Sept.</td>
<td>1 wk.</td>
<td>8</td>
<td>12</td>
<td>No</td>
<td>No</td>
<td>6</td>
<td>20 Mil.</td>
<td>7 days</td>
<td>5 wks.</td>
<td>5 wks.</td>
</tr>
<tr>
<td>12</td>
<td>14 mo.</td>
<td>Sept., Oct.</td>
<td>2 wks.</td>
<td>12</td>
<td>16</td>
<td>Yes</td>
<td>No</td>
<td>7</td>
<td>40 Mil.</td>
<td>3 days</td>
<td>20 days</td>
<td>3 wks. 2 days</td>
</tr>
<tr>
<td>13</td>
<td>30 mo.</td>
<td>Sept., Oct.</td>
<td>2 wks.</td>
<td>9</td>
<td>12</td>
<td>No</td>
<td>No</td>
<td>10</td>
<td>30 Mil.</td>
<td>3 days</td>
<td>6 wks.</td>
<td>3 wks.</td>
</tr>
<tr>
<td>14</td>
<td>8 mo.</td>
<td>Sept., Oct.</td>
<td>2 wks.</td>
<td>12</td>
<td>9</td>
<td>Yes</td>
<td>Yes</td>
<td>8</td>
<td>40 Mil.</td>
<td>3 days</td>
<td>9 days</td>
<td>4 wks. 1 day</td>
</tr>
<tr>
<td>15</td>
<td>6 yrs.</td>
<td>March, April</td>
<td>7 wks.</td>
<td>2</td>
<td>10</td>
<td>Yes</td>
<td>Yes</td>
<td>4</td>
<td>20 Mil.</td>
<td>7 days</td>
<td>35 days</td>
<td>5 wks. 4 days</td>
</tr>
<tr>
<td>16</td>
<td>30 mo.</td>
<td>February</td>
<td>5 wks.</td>
<td>6</td>
<td>4</td>
<td>Yes</td>
<td>Yes</td>
<td>5</td>
<td>40 Mil.</td>
<td>1 day</td>
<td>5 days</td>
<td>7 days</td>
</tr>
<tr>
<td>17</td>
<td>20 mo.</td>
<td>March</td>
<td>5 wks.</td>
<td>12</td>
<td>6</td>
<td>Yes</td>
<td>Yes</td>
<td>3</td>
<td>20 Mil.</td>
<td>3 days</td>
<td>8 days</td>
<td>5 wks.</td>
</tr>
<tr>
<td>18</td>
<td>9 mo.</td>
<td>April, May</td>
<td>5 wks.</td>
<td>20</td>
<td>12</td>
<td>Yes</td>
<td>Yes</td>
<td>4</td>
<td>20 Mil.</td>
<td>4 days</td>
<td>17 days</td>
<td>5 wks. 3 days</td>
</tr>
<tr>
<td>19</td>
<td>24 mo.</td>
<td>April, May</td>
<td>2 wks.</td>
<td>6</td>
<td>6</td>
<td>No</td>
<td>No</td>
<td>4</td>
<td>20 Mil.</td>
<td>6 days</td>
<td>3 wks.</td>
<td>3 wks. 4 days</td>
</tr>
<tr>
<td>20</td>
<td>7 mo.</td>
<td>May, June</td>
<td>5 wks.</td>
<td>12</td>
<td>3</td>
<td>Yes</td>
<td>Yes</td>
<td>3</td>
<td>20 Mil.</td>
<td>3 days</td>
<td>4 days</td>
<td>2 wks. 1 day</td>
</tr>
<tr>
<td>21</td>
<td>2 mo.</td>
<td>March</td>
<td>3 wks.</td>
<td>3</td>
<td>2</td>
<td>Yes</td>
<td>Yes</td>
<td>9</td>
<td>20 Mil.</td>
<td>3 days</td>
<td>3 wks.</td>
<td>3 wks. 3 days</td>
</tr>
<tr>
<td>22</td>
<td>30 mo.</td>
<td>March</td>
<td>5 wks.</td>
<td>4</td>
<td>3</td>
<td>Yes</td>
<td>Yes</td>
<td>6</td>
<td>40 Mil.</td>
<td>3 days</td>
<td>7 wks.</td>
<td>3 wks. 1 day</td>
</tr>
<tr>
<td>23</td>
<td>4 yrs.</td>
<td>March</td>
<td>4 wks.</td>
<td>24</td>
<td>24</td>
<td>Yes</td>
<td>Yes</td>
<td>8</td>
<td>40 Mil.</td>
<td>3 days</td>
<td>15 days</td>
<td>3 wks. 1 day</td>
</tr>
<tr>
<td>24</td>
<td>3 yrs.</td>
<td>Jan., Feb.</td>
<td>5 wks.</td>
<td>12</td>
<td>24</td>
<td>Yes</td>
<td>Yes</td>
<td>10</td>
<td>20 Mil.</td>
<td>3 days</td>
<td>12 days</td>
<td>4 wks. 4 days</td>
</tr>
<tr>
<td>25</td>
<td>2 yrs.</td>
<td>Jan., Feb.</td>
<td>4 wks.</td>
<td>24</td>
<td>4</td>
<td>Yes</td>
<td>Yes</td>
<td>7</td>
<td>40 Mil.</td>
<td>3 days</td>
<td>14 days</td>
<td>2 wks. 3 days</td>
</tr>
<tr>
<td>26</td>
<td>4 yrs.</td>
<td>April, May</td>
<td>4 wks.</td>
<td>12</td>
<td>4</td>
<td>Yes</td>
<td>Yes</td>
<td>6</td>
<td>40 Mil.</td>
<td>3 days</td>
<td>18 days</td>
<td>3 wks.</td>
</tr>
<tr>
<td>27</td>
<td>6 yrs.</td>
<td>January</td>
<td>2 wks.</td>
<td>6</td>
<td>2</td>
<td>Yes</td>
<td>Yes</td>
<td>4</td>
<td>40 Mil.</td>
<td>2 days</td>
<td>7 days</td>
<td>1 wk. 3 days</td>
</tr>
<tr>
<td>28</td>
<td>3 yrs.</td>
<td>Jan., Feb.</td>
<td>5 wks.</td>
<td>8</td>
<td>5</td>
<td>Yes</td>
<td>Yes</td>
<td>11</td>
<td>40 Mil.</td>
<td>2 days</td>
<td>16 days</td>
<td>3 wks. 6 days</td>
</tr>
<tr>
<td>29</td>
<td>2 yrs.</td>
<td>May, June, July</td>
<td>2 wks.</td>
<td>8</td>
<td>6</td>
<td>Yes</td>
<td>Yes</td>
<td>6</td>
<td>20 Mil.</td>
<td>6 days</td>
<td>28 days</td>
<td>6 wks. 5 days</td>
</tr>
<tr>
<td>30</td>
<td>1 yr.</td>
<td>May, June</td>
<td>2 wks.</td>
<td>6</td>
<td>4</td>
<td>Yes</td>
<td>Yes</td>
<td>6</td>
<td>40 Mil.</td>
<td>2 days</td>
<td>9 days</td>
<td>2 wks.</td>
</tr>
<tr>
<td>31</td>
<td>1 yr.</td>
<td>July</td>
<td>6 wks.</td>
<td>20</td>
<td>24</td>
<td>Yes</td>
<td>Yes</td>
<td>6</td>
<td>40 Mil.</td>
<td>3 days</td>
<td>10 days</td>
<td>4 wks.</td>
</tr>
<tr>
<td>32</td>
<td>3 yrs.</td>
<td>May, June</td>
<td>5 wks.</td>
<td>6</td>
<td>12</td>
<td>Yes</td>
<td>Yes</td>
<td>9</td>
<td>40 Mil.</td>
<td>2 days</td>
<td>2 days</td>
<td>4 wks.</td>
</tr>
<tr>
<td>Case No.</td>
<td>Age</td>
<td>Month of Year Sick</td>
<td>Duration Before Vaccine</td>
<td>Cough</td>
<td>Vomiting</td>
<td>Vaccine</td>
<td>Doses, Interval</td>
<td>Vomiting Ceased</td>
<td>Duration Disease After Vaccine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>---------</td>
<td>--------------------</td>
<td>-------------------------</td>
<td>--------------</td>
<td>-------------</td>
<td>-------------</td>
<td>----------------</td>
<td>-----------------</td>
<td>-------------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>3 yrs.</td>
<td>Jan., Feb.</td>
<td>5 wks.</td>
<td>Day 12</td>
<td>Night 24</td>
<td>Yes</td>
<td>Yes</td>
<td>7</td>
<td>40 Mil.</td>
<td>14 days</td>
<td>4 wks. 4 days</td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>5 mo.</td>
<td>Feb., March</td>
<td>1 wk. 12 con.</td>
<td>30</td>
<td>30</td>
<td>No</td>
<td>No</td>
<td>10</td>
<td>50 Mil. 2&amp;3 days</td>
<td>3 wks. 3 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>3½ yrs.</td>
<td>March, April</td>
<td>1 mo.</td>
<td>24</td>
<td>24</td>
<td>Yes</td>
<td>Yes</td>
<td>10</td>
<td>50 Mil. 2&amp;3 days</td>
<td>31 days</td>
<td>4 wks. 3 days</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>5 yrs.</td>
<td>March, April</td>
<td>1 mo.</td>
<td>24</td>
<td>24</td>
<td>No</td>
<td>No</td>
<td>8</td>
<td>50 Mil. 2 days</td>
<td>2 wks. 6 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>3 yrs.</td>
<td>March, April</td>
<td>2 wks.</td>
<td>26</td>
<td>26</td>
<td>Yes</td>
<td>Yes</td>
<td>8</td>
<td>50 Mil. 2&amp;3 days</td>
<td>13 days</td>
<td>3 wks. 6 days</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>7 mo.</td>
<td>March, April</td>
<td>2 wks.</td>
<td>10</td>
<td>10</td>
<td>Yes</td>
<td>Yes</td>
<td>8</td>
<td>50 Mil. 2 days</td>
<td>10 days</td>
<td>3 wks. 3 days</td>
<td></td>
</tr>
<tr>
<td>39</td>
<td>4½ yrs.</td>
<td>March, April</td>
<td>2 wks.</td>
<td>28</td>
<td>28</td>
<td>Yes</td>
<td>Yes</td>
<td>8</td>
<td>50&amp;25 Mil. 2&amp;3 days</td>
<td>10 days</td>
<td>3 wks. 3 days</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>16 mo.</td>
<td>March, April</td>
<td>1 mo.</td>
<td>14</td>
<td>14</td>
<td>Yes</td>
<td>Yes</td>
<td>8</td>
<td>50&amp;25 Mil. 2&amp;3 days</td>
<td>6 days</td>
<td>3 wks. 2 days</td>
<td></td>
</tr>
<tr>
<td>41</td>
<td>4½ yrs.</td>
<td>April, May</td>
<td>1 wk.</td>
<td>24</td>
<td>10</td>
<td>No</td>
<td>No</td>
<td>5</td>
<td>50 Mil. 3 days</td>
<td>2 wks. 1 day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>3½ yrs.</td>
<td>April, May</td>
<td>2 wks.</td>
<td>(After 1st doses child stopped treatment 3 weeks)</td>
<td>24</td>
<td>14</td>
<td>Yes</td>
<td>Yes</td>
<td>7</td>
<td>50 Mil. 2&amp;3 days</td>
<td>35 days</td>
<td>2 wks. 5 days</td>
</tr>
<tr>
<td>43</td>
<td>1½ yrs.</td>
<td>March, April</td>
<td>1 mo. 1 wk.</td>
<td>24</td>
<td>24</td>
<td>Yes</td>
<td>Yes</td>
<td>9½</td>
<td>50 Mil. 2&amp;3 days</td>
<td>15 days</td>
<td>3 wks. 3 days</td>
<td></td>
</tr>
<tr>
<td>44</td>
<td>3 yrs.</td>
<td>May</td>
<td></td>
<td>4</td>
<td>4</td>
<td>No</td>
<td>No</td>
<td>4</td>
<td>40 Mil. 2 days</td>
<td>1 wk.</td>
<td>3 wks.</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>8 yrs.</td>
<td>May</td>
<td></td>
<td>5</td>
<td>5</td>
<td>No</td>
<td>No</td>
<td>1</td>
<td>40 Mil.</td>
<td>7 days</td>
<td>5 wks.</td>
<td></td>
</tr>
<tr>
<td>46</td>
<td>5 yrs.</td>
<td>May</td>
<td></td>
<td>6</td>
<td>6</td>
<td>No</td>
<td>No</td>
<td>2</td>
<td>20 Mil. 7 days</td>
<td>3 wks.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>47</td>
<td>3 yrs.</td>
<td>June</td>
<td></td>
<td>4</td>
<td>6-8</td>
<td>Yes</td>
<td>Yes</td>
<td>4</td>
<td>25-50 2 days</td>
<td>7 days</td>
<td>1 wk. 4 days</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>5 yrs.</td>
<td>June</td>
<td></td>
<td>8</td>
<td>4-6</td>
<td>Yes</td>
<td>Yes</td>
<td>4</td>
<td>50 Mil. 2-3 days</td>
<td>2 days</td>
<td>1 wk. 4 days</td>
<td></td>
</tr>
<tr>
<td>49</td>
<td>1 yr.</td>
<td>April, May</td>
<td></td>
<td>16</td>
<td>18</td>
<td>Yes</td>
<td>Yes</td>
<td>11</td>
<td>25-75 2-3 days</td>
<td>15 days</td>
<td>3 wks. 5 days</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>4 yrs.</td>
<td>May, June</td>
<td></td>
<td>10</td>
<td>6</td>
<td>Yes</td>
<td>Yes</td>
<td>3</td>
<td>50-100 6 days</td>
<td>2 days</td>
<td>1 wk. 4 days</td>
<td></td>
</tr>
<tr>
<td>51</td>
<td>13 mo.</td>
<td>May, June</td>
<td></td>
<td>20</td>
<td>24</td>
<td>Yes</td>
<td>Yes</td>
<td>9</td>
<td>25-50 2-3 days</td>
<td>2 wks. 5 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>52</td>
<td>3 yrs.</td>
<td>May, June</td>
<td></td>
<td>10</td>
<td>5-6</td>
<td>Yes</td>
<td>Yes</td>
<td>3</td>
<td>25-50 4-5 days</td>
<td>2 wks. 2 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>53</td>
<td>8 mo.</td>
<td>Oct., Nov.</td>
<td></td>
<td>8</td>
<td>7</td>
<td>No</td>
<td>No</td>
<td>2</td>
<td>100 Mil. 5 days</td>
<td>1 wk. 6 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>54</td>
<td>2 yrs.</td>
<td>Oct., Nov.</td>
<td></td>
<td>20</td>
<td>24</td>
<td>No</td>
<td>No</td>
<td>6</td>
<td>100 Mil. 2 days</td>
<td>2 wks. 4 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>55</td>
<td>10 yrs.</td>
<td>November</td>
<td></td>
<td>2-4</td>
<td>2-4</td>
<td>No</td>
<td>No</td>
<td>3</td>
<td>50 Mil. 2 days</td>
<td>1 wk.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>56</td>
<td>5½ yrs.</td>
<td>November</td>
<td></td>
<td>8-10</td>
<td>12-14</td>
<td>No</td>
<td>No</td>
<td>3</td>
<td>50 Mil. 3 days</td>
<td>11 days</td>
<td>1 wk. 4 days</td>
<td></td>
</tr>
<tr>
<td>57</td>
<td>18 mo.</td>
<td>October</td>
<td></td>
<td>4</td>
<td>6</td>
<td>No</td>
<td>No</td>
<td>6</td>
<td>25-50 3 days</td>
<td>2 days</td>
<td>2 wks. 3 days</td>
<td></td>
</tr>
<tr>
<td>58</td>
<td>6 yrs.</td>
<td>Sept., Oct.</td>
<td></td>
<td>16</td>
<td>22</td>
<td>No</td>
<td>No</td>
<td>9</td>
<td>50 Mil. 4-5 days</td>
<td>30 days</td>
<td>4 wks. 6 days</td>
<td></td>
</tr>
<tr>
<td>59</td>
<td>2 yrs.</td>
<td>October</td>
<td></td>
<td>6-8</td>
<td>4</td>
<td>Yes</td>
<td>Yes</td>
<td>5</td>
<td>50 Mil. 3-4 days</td>
<td>2 wks. 5 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>14 mo.</td>
<td>Dec., Jan.</td>
<td></td>
<td>6-8</td>
<td>5-6</td>
<td>Yes</td>
<td>Yes</td>
<td>8</td>
<td>50-150 2-5 days</td>
<td>10 days</td>
<td>3 wks. 3 days</td>
<td></td>
</tr>
<tr>
<td>61</td>
<td>6 yrs.</td>
<td>Nov., Dec.</td>
<td></td>
<td>5</td>
<td>3</td>
<td>Yes</td>
<td>Yes</td>
<td>10</td>
<td>50-100 2-7 days</td>
<td>16 days</td>
<td>4 wks. 4 days</td>
<td></td>
</tr>
</tbody>
</table>

Case 59 started treatment, had 2 injections, then stopped for 2 weeks, when treatment was resumed. Case 51 was complicated with broncho-pneumonia.
absence of night paroxysms and a reduction in the number of day paroxysms, so that by the end of a week or ten days the number of whoops is reduced to about a quarter of their previous frequency.

Preparation of Vaccine

The Bureau of Laboratories of the Health Department prepares the vaccine as follows:

A 48-hour culture on the special Bordet-Gengou medium is scraped off with normal salt solution, shaken three or four hours in the shaking apparatus, and heated for an hour to 60° C. (or left in an incubator over night at 56° C.) and standardized according to the Wright method, 0.25 per cent. carabolic acid being added as a preservative.

It will be seen by a perusal of the table that individual children responded differently to the pertussis vaccine, so that arbitrary rules as to dosage cannot be followed, but it seems perfectly safe to give an initial dose of 100,000,000 bacteria to a child under two years of age.

Subsequent doses may be larger, according to the requirements of the individual case. Children over two years of age may be given 200,000,000 bacteria at the first injection; subsequent injections should be given at from two to four days. In severe or obstinate cases it may be necessary to give the maximum or still further ascending doses several times, before definite clinical results are obtained. Clinical results seem to indicate that the vaccine given in fairly large or ascending doses brings about a more rapid cure than smaller doses of constant size.

The duration of whooping-cough under the older methods of treatment was variable, but usually lasted from eight to twelve weeks in the light, uncomplicated cases, but the more severe ones, these being the majority, lasted a considerably longer time.

Exacerbations often prolonged each of the different stages so that the disease not infrequently was of months' duration. With the use of pertussis vaccine we believe the duration of the disease to be shortened to almost half what it was when treated by drugs.

Vomiting, which is frequently such an annoying and serious symptom of whooping-cough on account of its exhausting effect, the child not being able to retain sufficient food for proper nourishment, is often quickly relieved by means of the vaccine.

Complications which have been so common under the older methods of treatment—bronchopneumonia, diarrhea, and convulsions being among the worst—have been of rare occurrence under the vaccine treatment, and when complications have occurred they have been of mild nature.

The data that have already been accumulated by numerous workers indicate that pertussis vaccine has a value in the treatment of whooping-
cough, particularly in the alleviation of symptoms and in shortening the course of the disease.

**VACCINE FOR PROPHYLAXIS**

Children in families in which other members are suffering from whooping-cough, or those who are otherwise liable to exposure, should be given immunizing doses of pertussis vaccine made from the Bordet-Gengou bacillus.

I have used this vaccine in 20 children, given in prophylactic doses of 25- to 50,000,000 bacteria, and in one child 75,000,000. The injections were given a week apart, and the children were given from one to three injections. These children were in families where other members were suffering from pertussis, and they were not isolated, but constantly exposed to the disease. They were closely observed from one to two months, and did not develop whooping-cough.

Hess (5) reports the prophylactic inoculation of 244 children in the Hebrew Infant Asylum, where there was an epidemic of pertussis. Of these, 20 developed the disease in from two and a half weeks to two and a half months or more, or one in twelve developed the disease; while of 80 children who were not vaccinated 59, or nearly three in four, developed whooping-cough.

When a child is already in the stage of incubation, the vaccine will not prevent the development of the disease, but where the child is not, but about to be exposed, several doses of the vaccine will act as a prophylactic and afford immunity for a certain length of time, at least two or three months, as we have shown; possibly, and probably, for a much longer time.

**VACCINE AS A MEANS OF REDUCING INFANT MORTALITY**

Whooping-cough, as we know, is distinctly a disease of infancy and early childhood. Figures covering a period of eight years at the Good Samaritan Dispensary show that of 934 patients treated, there were under one year 203, one to three years 375, three to five years 219, five to ten years 135, or about 84 per cent. occurring before the fifth year, while the first to the third year was the most susceptible age.

A report from the New York Health Department for the years 1908 to 1912 and eleven months of 1913 shows that there was a decrease in the number of deaths from measles, scarlet fever, and diphtheria, notwithstanding the increase in population, while the mortality from whooping-
cough has increased, showing that the methods of treatment up to the present time have not been effectual in decreasing the mortality to any appreciable degree in this disease.

Infancy is the most fatal time for this disease to develop, on account of the frequency of complications at this period.

The deaths from whooping-cough in the Greater City of New York during the years 1908 to 1912 inclusive were as follows:

<table>
<thead>
<tr>
<th>Year</th>
<th>Under 1 year</th>
<th>1 year</th>
<th>2 years</th>
<th>3 years</th>
<th>4 years</th>
<th>Total under 5 years</th>
<th>5-10 years</th>
<th>10-15 years</th>
<th>15 years and over</th>
</tr>
</thead>
<tbody>
<tr>
<td>1908</td>
<td>113</td>
<td>48</td>
<td>16</td>
<td>5</td>
<td>2</td>
<td>184</td>
<td>3</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>1909</td>
<td>206</td>
<td>105</td>
<td>36</td>
<td>28</td>
<td>11</td>
<td>386</td>
<td>12</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>1910</td>
<td>156</td>
<td>72</td>
<td>29</td>
<td>16</td>
<td>6</td>
<td>281</td>
<td>11</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1911</td>
<td>203</td>
<td>101</td>
<td>34</td>
<td>22</td>
<td>14</td>
<td>374</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1912</td>
<td>151</td>
<td>76</td>
<td>28</td>
<td>12</td>
<td>6</td>
<td>273</td>
<td>12</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>829</td>
<td>402</td>
<td>143</td>
<td>85</td>
<td>39</td>
<td>1,498</td>
<td>48</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

This table shows that from 50 to 60 per cent. of the deaths were in children under one year, and about 80 per cent. of the deaths were in children of one year and under.

During the year 1911 there were 6,682 deaths from whooping-cough in the registration area of the United States, and of these 3,687 were under one year, while 1,611 were one year of age. About 80 per cent. of the fatal cases were under two years of age, while over 50 per cent. were under one year.

Although it would be a difficult matter to show, by statistics, the mortality from whooping-cough, since all cases are not reported, many mild ones being missed and a few severe ones not diagnosed or concealed, yet we know that in the first year of life the mortality is high, probably reaching 20, 30, or even 40 per cent., especially during severe epidemics and in foundling asylums and hospitals.

After the first year the mortality diminishes rapidly. Thus Vienna statistics (7) for the year 1899 to 1901 show that of 1,242 cases reported during the first year of life 322, or 25.3 per cent., died, while in those reported from the second to the fifth year there was a mortality of 6.8 per cent.

In 1915, 6,868 cases were reported in New York, with 385 deaths, or a mortality of 5.60 per cent. When consideration is taken of unreported, atypical and adult cases the mortality is probably about one per cent. for the disease, all ages included. Thus the data accumulated under
the older methods of treatment all indicate that whooping-cough in infants under one year of age is a comparatively fatal disease. Of a considerable number of cases already reported by different workers treated by means of vaccine deaths have been rare. The number of cases treated with the pertussis vaccine is yet too small to make comparisons of the mortality of treated and untreated cases, but from the results so far obtained it would seem that by means of the vaccine, especially if given early in the disease before complications arise, we shall be able to reduce the mortality from this disease.

Pertussis presents several characteristics which suggest the possibility of favorable results from specific therapy. From the work of Bordet and Gengou and of Mallory we believe that the disease is caused by a small bacillus which occurs in large numbers among the cilia of the tracheal and bronchial mucous membrane. The disease persists for weeks; many of the severe symptoms result secondarily from mechanical causes, and the mortality is due largely to complications. One attack protects against subsequent attacks, and the serum of animals immunized by repeated inoculations of the causative bacillus, as well as the blood of patients suffering from the disease, contains specific agglutinating and complement-fixing substances.

The treatment of the disease by active immunization has been reported by a number of workers as outlined above, and the indications are that vaccines are of value in lessening the severity of the disease. Nicolle and Coñor treated 104 cases in an epidemic in Tunis. Cessation of cough with cure occurred in 35 per cent., improvement in 38 per cent., and in 26 per cent. the disease remained stationary. In the first group an early improvement after one or two inoculations was noted. Bamberger (1) reported six cases in which the inoculations appeared to lessen the severity of the disease and decrease complications rather than to shorten the course. Graham (4) collected records of 24 patients, seven of whom apparently derived no benefit from the inoculations. In the remainder it was thought that the vaccines were of some value. Saunders, Johnson, White, and Zahorsky (11) gave immunizing doses (three inoculations at intervals of nine to ten days) to 14 supposedly non-immune children in an institution in which whooping-cough was epidemic. Of these children one developed the disease with paroxysms for one week only, seven had cough of mild character without paroxysms, and six had no cough. All were repeatedly exposed to infection.

A further study of the results in inoculated individuals with special reference to prophylaxis, the amelioration of symptoms, the occurrence of complications, and relative mortalities is desirable before the exact value of active immunization in whooping-cough can be determined.

Serum Therapy.—Following the suggestions of Bordet, attempts have
been made to utilize immune sera (horse) in the treatment of pertussis. Klimenko (5) reported improvement in a number of patients thus treated. Duthoit (3) reported 72 cases treated by the immune serum of Bordet and Gengou and concluded that the serum exhibited no marked influence in promoting recovery from the disease. In the discussion of this paper Bordet expressed the opinion that there is apparently little hope of obtaining an effective antiserum in whooping-cough.—Editors.]

REFERENCES

3. Duthoit, Raoul. Soixante-douze cas de coqueluche traités par le
   serum Bordet-Gengou, Ann. et bull. de la soc. roy. d. sci. méd. et
   natur. de Bruxelles, 1912, lxx, 378.
   Jour. Dis. of Children, 1912, iii, 41.
6. Klimenko, V. N. Sur le serum anticoquelucheux et son emploi,
   1915, xvi, pp. 67-85.
12. Saunders, Johnson, White, and Zahorsky. Pertussis Vaccine as a
    Curative and Prophylactic Agent, Pediatrics, 1912, xxiv, 161.
CHAPTER XII

TUBERCULIN TREATMENT

LOUIS HAMMAN

The object of this article is to provide the practicing physician with a guide to tuberculin treatment. His needs, therefore, will be constantly held in mind, and, though it will be necessary from time to time to discuss scientific problems and somewhat abstract principles, such discussions will be indulged in only to the extent required to illuminate or explain practical application. Views about tuberculin treatment and the methods in vogue are far from uniform. These differences must be stated to put the interested physician in touch with the present position of the subject, and they must be discussed to justify the adoption of one rather than another method. However, the viewpoint of the physician will be sought, and the trend of the presentation flows always toward practice.

In accord with this object I must assume that the reader is inexperienced in the use of tuberculin, and particularly where methods are concerned give these in practical detail rather than in principle. No doubt details will be given that for many are unnecessarily minute, but I wish to leave no one in doubt just how to proceed. Wherever necessary illustrative material will be added to emphasize important points.

Everyone knows that there is widespread interest in tuberculin treatment and that it is gaining constantly in popularity, but few have stopped to review critically the evidence upon which its claims to serious consideration are based. Many physicians use tuberculin because it is popular, many because they have confidence in the opinion of prominent men who advocate it, and most, I dare say, because a conviction of its value has followed upon striking results observed in a few isolated instances. Perhaps the simplest way to preach the value of tuberculin is to persuade the unbeliever to use it and to allow conviction to follow experience. Still, the sceptic may refuse to be moved by such personal appeals and demand more consistent evidence. It is only fair to say that amidst the general acclaim of tuberculin there are a few dissenting voices, and these must be heeded.

It seems to me that the physician brought to face tuberculin treatment will ask, as his first question, what evidence have you that tuberculin is of
value? Before studying the elaborate principles of the treatment and the methods of application he will naturally wish to know the results that have been obtained. It is from this angle that we will approach tuberculin treatment.

THE RESULTS OBTAINED WITH TUBERCULIN TREATMENT

The evidence in favor of the value of tuberculin is voluminous and diverse, but unfortunately much of it is desultory. It is not a tempting task to review it in a systematic way. Most of the evidence upon analysis is reduced to impressions which, though of importance as bespeaking a good name for tuberculin, yet do not necessarily force conviction. There are inherent difficulties in statistical studies of tuberculosis that make it arduous to seek the evidence in that direction, and animal experiments have been far from satisfactory. It is impossible to consider in detail detached bits of evidence, so the published results will be taken up in groups with only a number of specific illustrations.

Animal Experiments.—We begin with animal experiments because, if there were satisfactory evidence in this direction, it would be the most conclusive obtainable and would make all further evidence superfluous. However, no such satisfactory evidence exists. Numerous authors have tested the value of tuberculin in the control of experimental infections in animals, and the consensus of opinion is that its influence is by no means striking. Almost constantly the treated animals live a short time longer than the untreated, but tuberculin has never stopped or even limited an established infection. More favorable claims than these have been asserted, but they have not stood the test of repetition. It is common to read in the literature that animals have been “immunized” with different varieties of tuberculin. Such statements are seldom accompanied by detailed protocols and do not bear a close scrutiny. Other observers never confirm the results. Indeed from Koch to the present day each inventor of a new kind of tuberculin cites animal experiments to sustain his claims to its superior virtues. These experiments are characterized usually by their small number, the paucity of detail with which they are reported, and a general indefiniteness of methods and results. Often the report consists merely of a statement. It is well to remember that real immunity or resistance to tuberculous infection has been obtained only with living tubercle bacilli.

While these statements present briefly a general conclusion from the whole mass of experimental work, many individual results are widely opposed. As is well known, Koch (78) based his original deductions partly upon animal experiments, experiments which were never subsequently confirmed. Beraneck (18) claims excellent results with his tu-
berrulin, stating that treated animals have outlived controls longer than a year. None of his animals were cured of the infection. Pearson and Gilliland (118) have attributed encapsulation of tuberculous lesions in cattle to tuberculin treatment. Trudeau (155) has demonstrated that experimental tuberculous of the eye in rabbits is favorably influenced by tuberculin. Sattler (141) and Zimmermann (175) obtained similar results. Baas (5) denies them. Baumgarten (15), Arlöing (2), Stroebe (153), and others failed to obtain any certain results, and recently Haupt (55) and Rabinowitz (131), using guinea-pigs and rabbits, come to the same conclusion. Pfuhl (141), Kitasato (74), and Spengler (149) report experimental tuberculosis benefited by tuberculin, while Czaplewski and Roloff (29) say their treated animals died quicker than controls. Denys (3) discards entirely animal experiments as a proof of the value of his tuberculin, claiming that animal experiments are unsuited to establish the worth of tuberculin.

We must admit that this attitude of Denys is not altogether unreasonable. While it would be a great comfort to have tuberculin treatment established firmly upon an experimental basis, still the absence of conclusive results in animals does not settle the question of its value. Experimental infection in animals and acquired infection in man are different aspects of the disease, and the value of tuberculin must rest ultimately upon the clinical results of its administration.

Clinical Results.—Clinical Impressions.—In speaking of the clinical results of tuberculin treatment, we shall refer temporarily only to pulmonary tuberculosis, since the evidence adduced pertains almost exclusively to this, the most widespread type of the infection. Later we shall offer the available evidence that concerns other forms of the disease. Regardless of Koch’s injunction that tuberculin was to be used only in early and moderately advanced stages of pulmonary tuberculosis, the remedy after its introduction was applied recklessly in all stages of the disease. Naturally enough the majority of the patients were hopelessly advanced. As was then the custom, large doses were administered, and it is shocking to glance at the clinical charts preserved from these days. Patients racked by a long illness and consumed by the fever of rapidly advancing disease were obliged to endure daily violent chills and the distressing symptoms characteristic of a severe tuberculin reaction. The absolute failure of tuberculin under these conditions to accomplish the promised results led to a profound reversal of feeling. The disappointment was so keen and the memory remaining so bitter that the weight of more recent conservative work has failed to overbalance the repugnance left in the minds of many physicians. The doom of tuberculin was sealed by the statement of Virchow that anatomical studies forced him to the belief that tuberculin treatment occasioned a mobilization of tubercle bacilli and a spread of the disease.
This question of mobilization of tubercle bacilli by tuberculin has constantly recurred in tuberculin literature. Following Virchow's pronouncement the question was investigated clinically and experimentally. Liebmnn (99) found tubercle bacilli in the blood of 56 patients in a group of 141 treated with tuberculin. Kossel (84) obtained only questionable results in examining 800 preparations. He was allowed to review Liebmann's slides, and concluded that Liebmann's results were due to faulty technique. Ehrlich and Guttmann (36), Petruschky (121), Trudeau (156), and others obtained only negative results. Baldwin (9) was unable to induce mobilization of bacilli by injecting tuberculin into tuberculous animals.

At present there is a lively discussion in medical literature concerning the presence of tubercle bacilli in the circulating blood of patients with pulmonary tuberculosis. Some authors, e.g., Kurashige (92), find them microscopically in all cases, whether early or late, while others are unable to find them in the most advanced cases. Animal experiments are likewise contradictory in result, some authors, e.g., Kennerknecht (73), obtaining constantly positive results, others, e.g., Liebermeister (100), a high percentage of positive results, and still others, e.g., Elsaesser (38), constantly negative results. We have investigated the question both microscopically and by animal inoculation in a large number of cases of advanced pulmonary tuberculosis, and our results have been uniformly negative. One might explain away the microscopical results on the assumption that the organisms seen, though acid-fast, are not tubercle bacilli. Brem (24) has demonstrated acid-fast bacilli in distilled water. The animal inoculations, however, are not so easily disposed of. The matter is hopelessly confusing. It is referred to here because so experienced an investigator as Lydia Rabinowitsch (131) avows that, while tubercle bacilli are usually absent from the blood of tuberculous animals, still they can be found as a rule after tuberculin injections. Bacmeister (7) has obtained similar results in man. The blood of 15 patients with phthisis was injected into animals; none of the animals developed tuberculosis. The patients were then subjected to a tuberculin reaction and blood withdrawn at the height of the reaction was injected into guinea-pigs. Four of the animals became tuberculous. The question is an important one and requires further study, but is so intimately bound up with the question of the occurrence of tubercle bacilli in the blood of tuberculous patients under ordinary conditions that the two must be solved together. As I have said, the answer to the latter question is at present inextricably confused, and I feel very strongly that it would be most unwise to draw premature clinical conclusions from the evidence at hand.

Although the early tuberculin era ended in disaster, still the results obtained at that time were not all unfavorable. Recently a prominent clinician (120) has written reminiscently of the immediate and per-
RESULTS OF TUBERCULIN TREATMENT

manent benefits of tuberculin treatment judged after the sobering interval of nineteen years. He was a physician at Davos, himself suffering from the disease, when the remedy was first introduced. Many observers felt that the downfall of tuberculin was occasioned by its indiscriminate and unreasoned application, and that perhaps a more cautious dosage would avoid the dangers while preserving the beneficial effects. As early as 1891 a number of prominent physicians—Ehrlich and Guttmann (36), Lichtheim (98), Aufrecht (3), Petruschky, and others—advocated the administration of small amounts and a cautious increase in dosage. Upon this plan many clinicians continued to use tuberculin, convinced that they were getting good results. We may mention especially Goetsch (47), Hager (50), Krause (85), Thorner (154), C. Spengler (150), Klebs (75), and Petruschky in Germany, and Trudeau (157) and von Ruck (161) in this country. In 1901 Goetsch published the first summary of the results of the treatment by this method upon a relatively large number of patients. These results received the endorsement of Koch, and from the time of their publication dates the modern era of tuberculin treatment. Numerous approving reports followed, and tuberculin rapidly gained a sure foothold as a method of treatment of recognized value. In 1905 and 1906 there were still many sceptics and some opponents, but during the past few years the verdict in its favor has become almost unanimous. In Germany the work of Petruschky (122), Turban (159), Moeller (109), Löwenstein (102), Sahli (8), Bandelier and Roepke (1) has served to bring it wide acclaim. In England Wilkinson (9), Wright (172), and Riviere and Morland (7) have rendered a similar service. In America Trudeau (157) and his pupils, chiefly Baldwin (9) and Brown (2), have given it wide popularity. In the face of this general approval a few consistent opponents have held out. The most prominent of these have been Meissen (105), Schroeder (146), Koehler (82), and Shaw (148). They have no data to prove that tuberculin is valueless, but they have exercised a rigid criticism of the evidence adduced in its favor. Enthusiasm has led many tuberculin champions to overstate its case and to draw unwarranted conclusions from ridiculously insufficient data. Their censorship has been of the greatest value in forcing us to recognize the worthlessness of many of the statistics upon which the value of tuberculin has been based, and to search for more convincing evidence.

Surely this mass of personal testimony in favor of tuberculin cannot be put lightly aside in forming an opinion. Many authors (Bandelier and Roepke, Riviere and Morland) consider it alone sufficient to force conviction, and seek no further evidence. However, to my mind, it has importance only by virtue of its mass, for the opinions taken separately, while founded upon experience, nevertheless, are supported for the most part by scant data. The character of these data must now receive our attention.
CLINICAL STATISTICS.—All statistical studies of pulmonary tuberculosis are surrounded with difficulties, and these difficulties are well-nigh insurmountable in a statistical study of methods of treatment. This statement takes into account the fact that there is no treatment that will cure tuberculosis. Methods of treatment may have more or less value, but the proof of their value is difficult to obtain, and just how valuable a method is generally eludes satisfactory expression. The statistics of tuberculin treatment upon which great store has been set are often upon analysis pitifully crude. The difficulty arises from the fact that in a disease of such long duration and such protean clinical manifestation, improvement and retrogression occur spontaneously in such an unpredictable way that the effects of treatment are hard to gauge. Standards of diagnosis are variable, and accurate classification for purposes of comparison is almost impossible.

Differences in diagnosis concern mainly early cases of pulmonary tuberculosis, but the moderately advanced group is to a limited extent involved. Too much emphasis has been put upon slight abnormalities in pulmonary physical signs in the diagnosis of pulmonary tuberculosis. Our studies\(^1\) have convinced us that many patients with quiescent lesions have been treated in sanatoria and now figure as cures in sanatorium statistics. I make this statement with confidence, since I have myself been guilty of the error. Whether it is or is not advisable to treat such cases in sanatoria is an open question, but that they should not be included in statistics of the results of treatment is obvious. That they enter as a serious disturbing factor in our estimate of the curability of pulmonary tuberculosis is certain. For example, C. Spengler (151), with bovine tuberculin, obtains 100 per cent. cures in stages 1 and 2 (Turban’s classification), with bovine and human tuberculin in 99.7 per cent. Such figures are beneath comment. Indeed I believe the factor to be so seriously disturbing that I lay little weight upon statistics of the results of treatment in closed pulmonary tuberculosis. Deductions would be far more convincing if only cases with tubercle bacilli in the sputum were included in such statistics. True, to enforce this demand would exclude from consideration a very important group of cases, but if there is no other remedy the lesser evil is to be preferred.

The difficulties of classification reside chiefly in the lack of correspondence between the extent of the disease and the severity of symptoms. A patient with very few physical signs may have rapidly progressing disease, while one with extensive physical signs may be in good condition, have no symptoms, and remain well indefinitely. Until the past few years Turban’s classification, based entirely upon the extent of pulmonary involvement, was the one in general use. More recently the National Association has proposed a schema which takes into account the physical

---

\(^1\) See Gellen and Hamman (58).
RESULTS OF TUBERCULIN TREATMENT

signs and the symptoms. This classification has been universally adopted in this country. In Germany a similar plan is in use which, however, differs from ours in some details, chiefly in the restriction of the incipient group. Although valuable as uniform plans for grouping cases, still they are far from satisfactory for rigid comparison; indeed, inherent difficulties make it impossible to propose a perfectly satisfactory classification. For instance, our moderately advanced group embraces widely different cases. One just missing the incipient group stands far apart from one just short of the advanced group. To these unavoidable difficulties investigators have added by following their own individual classifications. Many others disregard all classification and group their material in one lump, thus making it impossible to compare their results with any other data.

Although the classification of cases of pulmonary tuberculosis is inadequate, an estimate of the results of treatment is still more unsatisfactory. Personal impressions play a large part in the estimate. In a disease that requires years to bring about healing it is difficult to measure the influence of treatment that lasts six months. Most statistics that bear upon tuberculin treatment use as their standard of comparison the condition of the patient when treatment is begun as contrasted with his condition at its termination. During this period, however, tuberculin rarely is the only factor to be taken into account. Usually there are concomitant changes in the patient’s surroundings and mode of life that deserve equal emphasis. Leaving this consideration aside, there are still serious objections to the standard of comparison itself. Upon what shall the test of improvement rest? Changes in the physical signs are not a satisfactory measure of the patient’s improvement. It is notorious how persistent physical signs are, even when general improvement is marked. Again, though considerable healing may have occurred, the signs may show no diminution in extent, while, on the other hand, an area may have become more seriously involved and the signs still remain unchanged. Added to this is the difficulty of appreciating slight changes in physical signs, when a record written months before is the only source of comparison. Obviously wide latitude is thus given to personal interpretation.

Nor are the symptoms a safer guide. In all sanatorium patients, except the hopelessly advanced, symptomatic cure is the rule. That such symptomatic cure is untrustworthy evidence of the permanent value of treatment is shown by following patients after discharge from sanatoria. Unfortunately a large proportion soon relapses. From the condition on discharge one cannot predict which cases will relapse and which will permanently hold improvement.

These objections to tuberculosis statistics have been recognized by investigators who seek to put the value of tuberculin treatment upon a firm basis. Therefore they have sought more satisfactory standards of
comparison, and recently have proposed these standards: (1) working
ability; (2) the disappearance of tubercle bacilli from the sputum; (3)
duration of life. All three of these standards possess obvious advantages
over the condition of the patient on discharge. They are arranged in the
inverse order of their importance. While the working ability of the
patient or his relative earning capacity, which is often considered equiva-

tent, is a rough estimate of his condition, still the objection may be urged
that the working capacity as gauged and reported by the patient himself
will be influenced by social conditions and the individual's temperament.
The disappearance of tubercle bacilli from the sputum is an objective
fact shorn of all personal misinterpretations. Besides, since only patients
with tubercle bacilli in the sputum are admitted to the study, the diag-

nosis is assured in each case. The disappearance of tubercle bacilli is an
important indication of improvement, and if, under one method of treat-
ment, bacilli disappear more regularly and earlier than under another it
is a reasonable conclusion to assume that the method with the larger
proportion of disappearance has decided advantages. Lastly, most con-
vincing of all are statistics of life duration. This is the final and abso-
lute test of treatment. Unfortunately, such statistics are gathered with
great difficulty, and many years must elapse before the results are avail-
able.

It is evident that for tuberculin statistics to be of value a number of
rigid requirements must be followed. To equalize the personal factor the
cases should be studied by one man, or at least, in an institution with
continuous and permanent traditions. To overcome the influence of
spontaneous variation in the course of the disease a large number of
patients should be studied. Side by side with the group of tuberculin-
treated patients an equally large group of patients as nearly similar as pos-
sible should be observed under identical conditions, save that tuberculin is
withheld. As a method of evaluating the results of treatment, the disap-
pearance of tubercle bacilli from the sputum, the working ability, and the
duration of life are to be preferred to the condition of the patient on
discharge.

A desire to present the results of tuberculin treatment unembellished
has drawn me unwillingly into this lengthy preamble. However, fair-
ness demands some such consideration. It will be seen that in the light
of this criticism many statistical studies to which undeserved esteem has
clung dwindle into personal impressions. As personal impressions they
retain their just value. I hasten to give a few of the more important
statistical studies, believing that without further comment the reader
will be able to attach to them their real worth. Some of these studies
have more historical interest than intrinsic value. I state them briefly,
and those sufficiently interested to wish details must consult the original
publications.
Ehrlich and Guttmann (36) report marked improvement in patients treated with tuberculin. No control observations.

Langenbach and Wolff (94) treated 99 patients with tuberculin, and at the same time observed 99 patients as nearly as possible in the same condition without tuberculin. Of the treated 33 were healed and 21 died; of the untreated 9 were healed, 45 died.

Petruschky (123) reports 54 cases of closed pulmonary tuberculosis all healed under treatment, and 38 cases of open pulmonary tuberculosis, of which 15 were healed and 22 died.

Goetsch (47) reports the results of treatment in 224 patients. Eighty-eight had tubercle bacilli in the sputum and one in the glands; 135 reacted to subcutaneous injections of tuberculin. One hundred and twenty-five cases in the incipient stage were cured. The remaining 99 cases had been under treatment too short a time to give the results any value. Although these results have been given prominence in discussion upon the value of tuberculin treatment, their publication was followed at once by well-directed criticism from Petruschky.

Denison (32) reports excellent results in 213 patients. He prefers von Ruck's watery extract, and of 33 patients treated with it 28 were alive and 5 dead nearly 2 years after treatment.

Moeller (109) reported the first large comparative study of tuberculin treatment. His report is from the Belzig sanatorium, and the results are as follows:

<table>
<thead>
<tr>
<th>Stage, Tuberculosis</th>
<th>Number</th>
<th>Healed (per cent.)</th>
<th>Arrested (per cent.)</th>
<th>Improved (per cent.)</th>
<th>Unimproved or Failed (per cent.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>134</td>
<td>281</td>
<td>51</td>
<td>32</td>
<td>37</td>
</tr>
<tr>
<td>2</td>
<td>105</td>
<td>290</td>
<td>18</td>
<td>3</td>
<td>44</td>
</tr>
<tr>
<td>3</td>
<td>90</td>
<td>362</td>
<td>0</td>
<td>0</td>
<td>41</td>
</tr>
<tr>
<td>Totals</td>
<td>329</td>
<td>933</td>
<td>27</td>
<td>10</td>
<td>40</td>
</tr>
</tbody>
</table>

Hager (50) reports using tuberculin since 1890. Forty patients (out of what number not stated), with tubercle bacilli in the sputum, have remained well, and likewise 220 cases diagnosed by the tuberculin reaction.

Denys (3) reports in great detail the results of treatment in 442 patients all with tubercle bacilli in the sputum. He contrasts with these 35 untreated patients. The statistics were gathered over a period of five years. Of the treated patients 193, or 43.6 per cent., were cured; 56, or 12.6 per cent., arrested; 36, or 8.1 per cent., much improved; 39, or 6.5 per cent., improved; 19, or 4.2 per cent., stationary; 9, or 2 per cent.,
worse, and 100, or 22.6 per cent., dead. Of the 35 patients who refused treatment 4, or 11.4 per cent., cured; 2, or 5.7 per cent., remained stationary; 5, or 14.2 per cent., were worse; and 24, or 68.5 per cent., were dead. Of the 442 cases treated with tuberculin 193, or 43.6 per cent., lost tubercle bacilli from the sputum.

Schnoller (144) reports using Denys' tuberculin in 211 patients with the following results:

<table>
<thead>
<tr>
<th></th>
<th>1st Stage</th>
<th>2nd Stage</th>
<th>3rd Stage</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probably cured</td>
<td>17</td>
<td>30</td>
<td>2</td>
<td>49 (23.2%)</td>
</tr>
<tr>
<td>Greatly improved</td>
<td>6</td>
<td>65</td>
<td>34</td>
<td>105 (49.8%)</td>
</tr>
<tr>
<td>Improved</td>
<td>2</td>
<td>19</td>
<td>11</td>
<td>32 (15.2%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>25 (100%)</td>
<td>114 (94.2%)</td>
<td>47 (72.3%)</td>
<td>186 (88.2%)</td>
</tr>
</tbody>
</table>

Stationary, stages II and III, 16 cases; worse, stage III, 6 cases; dead, stages II and III, 3 cases. Of 148 patients 44, or 29.7 per cent., lost tubercle bacilli from the sputum.

Turban (159) treated 86 patients with tuberculin and contrasts them with 241 untreated patients. Permanent healing was obtained in 53 per cent. of the former and 39 per cent. of the latter.

Nagel (113) reports a large number of cases from the sanatorium at Cottbus. It is pertinent to note that but 15 per cent. of the patients had tubercle bacilli in the sputum. The study included patients in the sanatorium from 1900 to 1905. During the years 1900 and 1901 tuberculin was not used, and the results are contrasted with those of 1902 to 1905, when tuberculin was used.

<table>
<thead>
<tr>
<th>Result</th>
<th>I Stage</th>
<th>II Stage</th>
<th>III Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Per cent.</td>
<td>Number</td>
</tr>
<tr>
<td>A</td>
<td>111 (227)</td>
<td>53.2 (41.6)</td>
<td>0 (18)</td>
</tr>
<tr>
<td>BI</td>
<td>84 (278)</td>
<td>40.2 (50.3)</td>
<td>21 (109)</td>
</tr>
<tr>
<td>A BI</td>
<td>195 (505)</td>
<td>93.4 (91.3)</td>
<td>21 (127)</td>
</tr>
<tr>
<td>BII</td>
<td>7 (47)</td>
<td>3.3 (8.5)</td>
<td>28 (40)</td>
</tr>
<tr>
<td>A BI BII</td>
<td>202 (552)</td>
<td>96.7 (99.8)</td>
<td>49 (167)</td>
</tr>
<tr>
<td>C</td>
<td>7 (1)</td>
<td>3.3 (0.2)</td>
<td>34 (14)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>209 (553)</td>
<td>100</td>
<td>83 (181)</td>
</tr>
</tbody>
</table>

The figures in parenthesis represent the tuberculin-treated. A = Clinically healed. BI = Full working ability. BII = Partial working ability. C = No effect; worse.
RESULTS OF TUBERCULIN TREATMENT

Of 96 patients with tubercle bacilli in the sputum treated with tuberculin 48 per cent. lost the bacilli. Of 65 patients with tubercle bacilli in the sputum not treated with tuberculin 20 per cent. lost the bacilli.

Reliable statistics covering life duration are those published by Brown (2) from Saranac. His comments are as follows:

While the number of patients treated with tuberculin at the Adirondack Cottage Sanatorium has not been large, the care with which the patients have been followed renders the following results of interest. To allow of comparison, since the number in each group varied so much from year to year, it is necessary to reduce or increase the number of treated and untreated in each class each year to 100. This gives the following tables, expressed in percentages, in which are included the results on discharge and the ultimate results of 185 patients treated with and 864 treated without tuberculin who remained in the institution over ninety days and had tubercle bacilli in their sputum:

Results on Discharge

<table>
<thead>
<tr>
<th></th>
<th>With Tuberculin</th>
<th>Without Tuberculin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>INCIPIENT:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apparently cured</td>
<td>56</td>
<td>50</td>
</tr>
<tr>
<td>Disease arrested</td>
<td>34</td>
<td>38</td>
</tr>
<tr>
<td>Active</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td><strong>MODERATELY ADVANCED:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apparently cured</td>
<td>27</td>
<td>6</td>
</tr>
<tr>
<td>Disease arrested</td>
<td>55</td>
<td>51</td>
</tr>
<tr>
<td>Active</td>
<td>18</td>
<td>43</td>
</tr>
</tbody>
</table>

The ultimate results, expressed in percentages of those living one to fifteen years after discharge, proper allowance being made for the varying numbers in each year and class, are as follows:

Ultimate Results

<table>
<thead>
<tr>
<th></th>
<th>With Tuberculin</th>
<th>Without Tuberculin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>INCIPIENT:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apparently cured</td>
<td>88</td>
<td>78</td>
</tr>
<tr>
<td>Disease arrested</td>
<td>77</td>
<td>78</td>
</tr>
<tr>
<td>Active</td>
<td>33</td>
<td>27</td>
</tr>
<tr>
<td><strong>MODERATELY ADVANCED:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apparently cured</td>
<td>91</td>
<td>86</td>
</tr>
<tr>
<td>Disease arrested</td>
<td>48</td>
<td>45</td>
</tr>
<tr>
<td>Active</td>
<td>41</td>
<td>22</td>
</tr>
</tbody>
</table>
These statistics indicate that on discharge the incipient cases have done somewhat better than those receiving no tuberculin, while the moderately advanced cases show much better results. The ultimate results do not show such marked differences, but indicate that the treated, both incipient and moderately advanced, do better.

I now present the sputum statistics, figures which, from their objectivity and their almost indubitable meaning, are extremely valuable. They speak strongly for the healing effect of tuberculin.

Kremser (90) chose 110 patients expectorating tubercle bacilli, treating 55 of them with tuberculin. The patients were not selected, but were placed in the groups alternately as they were admitted. Of those treated with tuberculin 22, or 41 per cent., lost the bacilli; of those treated without tuberculin only 16, or 29 per cent.

Phillipi (126) finds that in his II stage cases 58 per cent. of those treated with tuberculin, against 19 per cent. of the untreated, were rid of bacilli in the sputum; and in the III stage cases 31 per cent. of the treated, as against only 7 per cent. of the untreated.

Turbán (159) reports that of 86 open cases treated by tuberculin 47.7 per cent. lost their bacilli; of 24 untreated only 27.4 per cent.

Brown (2) reports from Saranac that in the incipient cases 67 per cent. of the tuberculin patients were rid of bacilli; of the others 64 per cent. In the moderately advanced the figures are respectively 44 per cent. and 24 per cent.

Bandelier (11) reports 500 cases, of whom 202 had tubercle bacilli in the sputum. On discharge after an average treatment of five to six months, 129, or 64.9 per cent., had the sputum changed from positive to negative. Twelve were in stage I; of those 100 per cent. became negative. Of the 113 in stage III 50 per cent. became negative. Bandelier challenges the production of similar results without tuberculin, and says they are unparalleled in the literature. These figures are remarkable, yet they are based on a respectable number—202 cases.

It is important to note that these percentages are closely paralleled by those of E. Löwenstein (102), who quotes the gratifying number of 682 open cases. No case is reported that did not reach the dose of 10 mg. O. T. Four sputum examinations were required to establish a case as negative. Under the tuberculin treatment 361 of the 682 cases finally showed negative sputum—a percentage of 53. Such a result, he maintains, cannot be obtained in any other way than by tuberculin. His analysis of the results of twenty years of hygienic-dietetic cure without tuberculin gives only 15 per cent. of the discharged as having no bacilli in the sputum.

Bandelier has classified the 500 cases above referred to, containing 202 open cases, also from the point of view of working capacity. Compared with the sputum results, the figures are as follows:
### RESULTS OF TUBERCULIN TREATMENT

<table>
<thead>
<tr>
<th></th>
<th>Total Cases</th>
<th>Stage I per cent.</th>
<th>Stage II per cent.</th>
<th>Stage III per cent.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete earning capacity on discharge</td>
<td>500</td>
<td>69.8</td>
<td>90.4</td>
<td>80.7</td>
</tr>
<tr>
<td>Sputum changed from positive to negative</td>
<td>202</td>
<td>63.9</td>
<td>100.0</td>
<td>87.3</td>
</tr>
</tbody>
</table>

It is seen from the table that statistics based on the sputum becoming negative afford a real evidence of improvement, even when that is judged from the purely symptomatic side. The parallelism between the two sets of figures is close, and forms an additional argument for taking the bacillary content of the sputum as a statistical basis.

Thus far I have spoken only of the results of tuberculin treatment in pulmonary tuberculosis. Favorable reports of treatment in so-called surgical forms of the disease are no less numerous. However, the number of cases treated by any one observer is small, and as far as I know there are no large statistical studies of parallel groups of cases. However, I have already emphasized that such personal evidence, though not strictly objective, is still of value, and its value is considerable when, as is indeed the case, the number of favorable reports is constantly on the increase and hostile publications almost completely disappear. External forms of tuberculosis are particularly favorable for estimating the effects of tuberculin, and I may say that many ophthalmologists, for instance, are among its most ardent advocates. I will not give the published results in detail. Space does not permit, and those interested may seek further information in the original articles.

Tuberculous laryngitis is not a thankful field for therapeutics by any method of treatment. It is usually a concomitant of advanced pulmonary tuberculosis and, when ulcerative lesions of the larynx are present, seldom permanently heals. In the infiltrative stage the prognosis is by no means bad, and many observers look upon tuberculin as the most effective agent in checking the advance of the disease. In the early tuberculin era Gerhardt (46) and B. Fraenkel (40) report good results. P. Krause (86) and Moritz are later enthusiasts. Willers, Polaczek, Wilkinson (9), Williams and Wagget, F. Krause, Ed. Meyer (106), F. Meyer (107), Bandelier and Roepke (1), Sahli (8), Grant and Watson-Williams (48) are adherents of the method.

Tuberculous lesions of the eye are among those that have in our experience responded most satisfactorily to tuberculin treatment. I recall several cases of tuberculosis of the cornea and iris that cleared up in an astonishing manner under tuberculin after they had resisted for months other methods of treatment. Reports of excellent results have been pub-
lished by v. Hippel (62), Saathoff, Griffith, Davids (31), Scheuermann (142), Schoeler (146), Ullman, Cramer, Augstein, Hornicker, Weeks (164), Lichtenstein, Erdmann, Tardieu, Schieck, Schaudigel, Herrenschwand (61), Heine (59), and Junius (69).

Numerous investigations commend the use of tuberculin in tuberculous adenitis upon the basis of excellent results observed in a large number of cases. Petruschky, Bauer, and Engel (13), Pagal, Jochmann (68), Heubner, Baginsky, Wright, Hawes and Floyd (56), Ager (1), Wilms (169), Hall, Philip (125), Griswold (49), Dautwiz (30), Kramer, Neumann (114), Scherer and Stoll (152) testify to its value.

In tuberculosis of bones and joints Kramer, Lenzmann (96), Jochmann (68), Schlossmann and Engel, Power, Raw (132), Neumann (114), Lüdke, Klose (77), Seyberts (147), Bungart (25), Jones, Beasley (16), F. Meyer (107), Bandelier (12), Smith and Cathcart report favorable results.

Most surgeons advise removal of the kidney when renal tuberculosis is early and involves only one organ. However, many of them advise post-operative treatment with tuberculin. Israel, Knorr, Casper (28), and Karo (71) may be mentioned. Whiteside (166), Bachrach and Necker (6), Bevan (20), and Kummell (91) advise its restricted use. Wildbolz (168), Douglas and Pedersen (119) report good results in renal cases. Frank (41) has seen no good results. In tuberculosis of the bladder and testicle and prostate gland Schulze, Jochmann (68), Mantoux (103), Lenhart, Pogue, Pardoe (116), Schroeder, Korntfeld, Sahli (8), Lenzmann (96), Ullman, Kehl (72) have used tuberculin. Birnbaum (21) has had striking success in tuberculosis of the female adnexa. Proschownik (130) and Kolischer (83) report favorable results in tuberculosis of the female genitalia.

In serous membrane tuberculosis many find tuberculin of value. Vernet (160) claims to have cured a case of tuberculous meningitis. In tuberculous peritonitis the reports are particularly favorable. Birnbaum (21) has abandoned operation in favor of tuberculin. Heiman (58), Olshausen, Zweifel, Fritsch, Ganghofer (44), Raw (132), and Denys (3) have had excellent results.

This mass of evidence shows very strikingly what a large number of advocates tuberculin has, and the statistical studies will point, with whatever weight may be attached to them, toward its value. From a consideration of this evidence the following conclusions seem to be warranted: Tuberculin is not a cure for tuberculosis, else such a detailed consideration were unnecessary. However, in many instances it promotes healing, and recovery is more certain and more lasting than without it. Such a conservative estimate of its influence ranks tuberculin as a favorable factor in the management of the disease, a favorable factor as rest and diet and fresh air are favorable factors. This being its position, it behooves
us to give it a wide application, but not to use it to the exclusion of other favorable factors. It should be employed in combination with these. It must be understood that tuberculin cannot replace fresh air or rest or diet in the treatment of tuberculous infections, and that we will do more harm than good if we make its use an excuse for relaxing our vigilance in respect to other important measures.

THE SELECTION OF A TUBERCULIN PREPARATION

We may reasonably assume that the evidence adduced in the previous section has stimulated the interested physician to look further into the subject of tuberculin treatment, and perhaps has created the desire to test its value himself. If such be his intention his next step will be to choose the tuberculin he wishes to use. Unfortunately the beginner is at once bewildered and discouraged by the large number of preparations offered him to choose from. Each product has its champion who proclaims its virtues superior to those of other tuberculins, and urges in support of these claims theoretical considerations and clinical results. I hope that the remarks made upon clinical deductions in estimating the value of any treatment in tuberculous disease will encourage the physician to review the alleged results critically. In view of recent investigations the whole question, at first so complicated, has become severely simple. But before stating the nature and results of these investigations we must give a brief statement of the composition and preparation of some of the most important tuberculins.

All the tuberculins may be divided roughly into three groups: (1) those prepared from the culture media in which tubercle bacilli have grown; (2) those prepared from the tubercle bacilli themselves; (3) those prepared by various methods of extracting the tubercle bacilli.

I may say briefly that all varieties of tubercle bacilli have been used in preparing tuberculins. Human type, virulent and avirulent; bovine type, virulent and avirulent; avian and piscian tubercle bacilli. Also that innumerable variations in culture media have been introduced. Only a few of the variations have acquired any permanent importance.

The principal members of group 1 are these:

Koch's (11) Original or Old Tuberculin: O. T.—Preparation.—A bouillon medium enriched with 5 per cent. glycerin and slightly alkaline is inoculated with tubercle bacilli of the human type. In a broad flask this is allowed to incubate at body temperature for six to eight weeks, at the end of which time the bacilli have grown into a flat sheet covering the surface of the fluid. Moistened fragments of the growth may have reached the bottom of the flask or may still be suspended at various depths. The entire contents are then subjected to a current of steam over a water
bath for the purpose of sterilization, and for concentration into one-tenth of the original volume. The glycerin, not evaporating, thus constitutes 50 per cent. of the resulting mixture. At this stage the bacteria (which have now been killed) are removed by filtration through a Chamberlain filter. There results a clear brown fluid of a characteristic odor, which keeps indefinitely and is ready for use.

Denys' (3) Bouillon Filtrate: B. F.—Preparation.—The culture is prepared as for making original tuberculin (O. T.). At the end of the required interval, however, the mixture is not heated or concentrated in any way, but is at once passed through a bacteria-proof porcelain filter. The residue is rejected. The filtrate, a clear fluid, is supposed to contain only the soluble secretions of the bacilli, plus the metabolized culture media, and without any further modification is ready for use.

Jochmann's (68) Albumose-free Tuberculin: A. F.—Preparation. —Following the lead of Proskauer, Beck, and Fraenkel, Jochmann grew tubercle bacilli on a protein-free medium made of water, 1,000; asparagin, 8; ammonium lactate, 6; sodium chloride, 5; glycerin, 40; neutral sodium phosphate, 2. From this culture fluid Jochmann prepares tuberculins which he deems less toxic, but therapeutically not more efficient than those tuberculins derived from the usual mediums. One of these is known as tuberculin A. F. (albumose-free). A. F., unlike O. T., is heated only to 37° C., and is concentrated to only 25 per cent. of the original volume. Tuberkulin Hell is heated to 100° C. Jochmann's clinical work was done largely with A. F.

Other members that may be mentioned are: Tuberculocidin (78), iron tuberculin (34), Endotin (43), Jessen's (67), Leber and Steinharter's (95); Calmette's Cl. (26).

The principal member of group 2 is:

Koeh's Bacilli-emulsion (126): B. E.—Preparation.—This, as the name indicates, is an emulsion of tubercle bacilli. The culture is grown as for O. T. The bacilli are filtered off, ground, but not washed. One part of the pulverized material is emulsified in 100 parts of distilled water, and an equal volume of glycerin added, making 50 per cent. glycerin emulsion, 1 c. c. of which contains the immunizing substance of 5 mg. of dried tubercle bacilli.

Other members that may be mentioned are v. Behring's preparations, Tulaselaktin and Tuberkulase (17), Tebean (97), Tuberculo-Sero-Vaccine of Meyer-Ruppel (108).

The principal members of group 3 are:

Koeh's Tuberculin-residue or New Tuberculin: T. R. (130).—Preparation.—Highly virulent cultures as young as possible are grown. After four to six weeks the bacilli are filtered off and dried in a vacuum. One gram of the dried tubercle bacilli is ground in an agate mortar until a sample shows no intact bacilli. To the pulverized mass is added 100
c. c. of distilled water, and the mixture is then centrifugalized. The clear fluid resulting from this centrifugalization is poured off and is known as Tuberculin Oberes (T. O.). It contains substances not precipitable by glycerin. The sediment deposited by centrifugalization is again dried, powdered, and again taken up by a small quantity of water. Centrifugalization is repeated and the previous cycle again gone through, until there is no sediment except that composed of gross, accidental particles. The fluids resulting from all the centrifugalizations, except the very first, are united, and should total not more than 100 c. c. This fluid is slightly opalescent and is precipitable by 50 per cent. glycerin. To the opalescent fluid 20 per cent. glycerin is added for preservation. The resulting suspension is known as T. R., and it should contain in each cubic centimeter 2 mg. of solids, representing 10 mg. of dried tubercle bacilli. From the mode of manufacture it was assumed that T. R. contains none of the secretions of the bacilli as does O. T., and that it does contain substances from the body of the bacilli, which O. T. speciously does not contain.

**Beranecck’s Tuberculin (19).**—Preparation.—In 1903 Beranecck announced a tuberculin for which he claims only minimal toxicity and a high content of specific substances. He cultivates the bacilli on a non-peptonized 5 per cent. glycerin bouillon medium which is not neutralized. The filtrate from this culture is known as T. B., or toxin-bouillon. The residue is shaken for a long time at 60 to 70° C. with 1 per cent. orthophosphoric acid. Equal volumes of the unheated toxin-bouillon and of the orthophosphoric acid extract of the bacillary bodies are united to form Beranecck’s Tuberculin of a concentration known as H.

**Von Ruck’s Watery Extract (49).**—Preparation.—Concentrate a culture in vacuo at 55° C. to 1-10 volume. (This takes about a month.) Filter through paper, then through porcelain. Precipitate with an acid solution of sodic-iodid of bismuth. Filter and neutralize the acid solution. Filter again. Precipitate with absolute alcohol to make 90 per cent. alcohol, and filter. Wash the precipitate with absolute alcohol. Dry the precipitate and make a 1 per cent. aqueous solution. Filter. The last filtrate is von Ruck’s tuberculin.

**Landmann’s Tuberculol (83).**—Preparation.—Landmann believed that in the process of heating O. T. to 100° C. substances are destroyed that at lower temperatures can be extracted. In order to obtain not only those extractives that cannot withstand heat, but also those that cannot be extracted without heat, he uses fractional extraction at various temperatures. He grows in bouillon a highly virulent strain of the human type of the tubercle bacillus. The bacilli are filtered off by filter-paper, fragmented, and the fatty components removed. Extraction at 40° C. then occurs by a glycerin-normal salt solution. After decantation the residue is again extracted at 50° C. and so up to 100° C. The united extracts are now concentrated in vacuo at 37° C. In order to make the aggregation
of tuberculous toxins still more complete the concentrated culture fluid is
now added to the combined extractives; and the entire amount is filtered
through porcelain for sterilization. Finally $\frac{1}{2}$ per cent. phenol is added.
The product is marketed by Merck as Tuberculol A.

In this group may also be mentioned oxtuberculin (63), iodin
tuberculin (7), tebesapin or proesperol (174), tuberculonastin (112),
and the tuberculins of Rosenbach (139), Buchner and Hahn (51),
Deycke and Much (33), and Ishigami (65).

It will be seen from the above list, incomplete though it is, that there
has been a feverish strife to improve old tuberculins and to produce ever
new and better tuberculins. Two considerations have prompted these
efforts:

1. The attempt, under the assumption that they are many, to include
all of the potent portions of the tubercle bacillus in the preparation.

2. The attempt to remove supposed deleterious substances from the
culture media or the bacilli themselves, while preserving uninjured the
beneficial or immunizing substances.

The first consideration was based upon principles of immunity estab-
lished for other diseases and transferred without warrant to tuberculosis.
As is well known, bacteriologists have distinguished two different poison-
ous substances obtained from bacteria: (1) exotoxins, or toxins secreted
by the organisms and present in the culture media, and (2) endotoxins,
or toxins intimately bound up with the living protoplasm of the bacteria
and liberated only upon their disintegration.

Exotoxins are probably a product of bacterial metabolism, and their
distinguishing features are their primary toxicity and the readiness with
which they stimulate in the animal organism the production of neutraliz-
ing bodies called antitoxin.

Endotoxins are intimately bound up with the living protoplasm of
bacteria, and are liberated when the organisms are disintegrated by cer-
tain ferment or lytic substances within the body. Although it is claimed
that antitoxins to endotoxins have been obtained, their appearance is at
least exceptional, and in general it is correct to say they produce no anti-
toxin.

Until recently it was customary to look upon tuberculous infec-
tions as producing specific secretions primarily toxic to the body. The
symptoms of intoxication so common in the disease—fever, loss of weight,
digestive disturbances, etc.—were looked upon as the direct effects of this
toxin. To this toxin it was supposed the body reacted by the production
of antitoxin, and the presence or absence of general symptoms depended
upon the balance existing between the two. However, though the toxin
might be completely neutralized and general symptoms be absent, still
the tubercle bacilli in the tuberculous lesion might continue to live and,
indeed, to multiply and to spread. The antitoxins therefore had no effect
upon the bacteria. To inhibit their growth the body must elaborate antibacterial substances, the production of such substances being a response to the stimulation of the bacteria themselves. It was concluded that in order successfully to combat tuberculous infections we must stimulate the body artificially to produce both antitoxins and bacteriolysins. Since toxins are soluble they must, of course, be present in the culture media, and broth filtrates were used to produce antitoxins. The bacteria themselves must be injected if we hope to reach any degree of antibacterial immunity.

It was these considerations that led Koch to prepare his different tuberculins. In his earliest experiments Koch observed that subcutaneous inoculations of tubercle bacilli in tuberculous guinea-pigs tended to prolong the life of the animals. However, necrosis and sloughing followed such inoculations, making the method impracticable for man. Following the established views of that day, Koch believed the healing effect of the injections to be due to diffusible substances, toxins secreted by the bacilli, and to avoid the necrosis used the broth filtrate instead of the bacilli themselves. Experience showing that, though the filtrate had a favorable influence upon the disease, still it did not satisfactorily control its progress, Koch once more turned to the bacillary bodies to obtain antibacterial immunity. The bacilli were ground up to prevent the occurrence of the necrosis that follows injections of whole organisms and the products called tuberculin-residue or T. R. and bacillen-emulsion or B. E. Furthermore, to obtain the full immunizing value of tuberculin he advised combining a filtrate and the bacillary body, for example, O. T. and B. E.

Such reasoning is not in accord with the latest views upon the nature of tuberculous infection and the mode of action of tuberculin. We know little directly about the endotoxins of tubercle bacilli, but nothing about the soluble toxins they are supposed to secrete. Indeed, all of the evidence we have accumulated about tuberculin goes to prove that the tubercle bacillus produces no true toxin. The experiments of Wassermann and Bruck (162) come nearest to demonstrating the presence of such toxins. From complement-absorption experiments they conclude that in tuberculous foci a substance is present that has a specific affinity for tuberculin. This antituberculin, as they call it, is found in the blood serum of patients only when tuberculin is being administered. Morgenroth and Rabinowitsch (110) failed to corroborate the results of Wassermann and Bruck, and Noguchi denies the specificity of the reaction. The results of the application of the test are so variable that no safe conclusions can be drawn from them. Even though we admit the presence of certain bodies in the blood that unite with tuberculin and absorb complement, still this admission does not stamp these substances as antitoxins. Single or repeated injections of large or small amounts of tuberculin never produce antitoxins in a healthy animal, nor do they cause antituberculin to appear
in the blood. Pickert and Löwenstein (127) claim that the serum of tuberculous patients who, under tuberculin treatment, have developed a high grade of tuberculin tolerance inhibits the cutaneous reaction when mixed with tuberculin. White and Graham (165) confirm these observations. However, Pickert and Löwenstein admit complete failure to neutralize the activity of tuberculin injected subcutaneously into guinea-pigs. The investigations of Römer (134) throw doubt upon the whole matter.

We know too little about the constitution of tuberculin to identify it by any chemical test. There is only one characteristic of tuberculin that is absolutely specific, namely, its power to produce a certain reaction in tuberculous animals. The features of this reaction are well known, and will be considered in detail later. Briefly, they are redness and swelling at the point of injection, inflammatory reaction about the lesion, and fever and other constitutional symptoms. Recent investigations have shown conclusively that the potent substance in tuberculin, the substance that causes this reaction, is the protein of the tubercle bacillus. This protein produces qualitatively always an identical reaction, whether the culture fluid be used, the bacilli themselves, or the pure protein extracted from the bacilli. A product containing this protein is a tuberculin, and no substance that does not contain it can be so classified. There is no other characteristic mark of a tuberculin.

Wolff-Eisner (170) has emphasized this point. He has worked with tuberculin which was shown microscopically to contain numerous acid-fast tubercle bacillus particles. Passed through a Chamberland or Berkefeld filter, the filtrate is found free from such particles, and still it produces reactions identical with, although weaker than, those of the original unfiltered product.

Tubercle bacillus protein being the potent constituent of tuberculin and, according to modern evidence, the only potent constituent, therefore any tuberculin that contains the specific protein is a satisfactory tuberculin to use. This at once settles the discussion about the value of the many different tuberculins. They are all satisfactory tuberculins if they contain tubercle bacillus protein, and the test of the presence of the protein is their ability to produce a tuberculin reaction. I emphasize this point since one reads constantly in the literature, and particularly in advertising literature, that this or that tuberculin is to be preferred because it has been rid of reaction-producing substances while the immunizing substances have been retained. According to our present views the reaction-producing and immunizing substances are one, and to free a tuberculin of its power to produce a reaction in the tuberculous is to rob it of the substance that gives it value in treatment. Other tuberculins are urged as superior upon the ground that they are primarily more highly toxic than other tuberculins. This is the sole argument in favor of, for in-
stance, tuberculol. But it must be evident from what has gone before that this claim has no substantial value.

Many authors contend that the specific constituents of tuberculin are more potent when subjected to the least possible amount of manipulation. They object to heat particularly, fearing that high temperatures may destroy or injure some of the constituents. This consideration led Denys to substitute B. F. for O. T. The argument is reasonable, but it is purely hypothetical. There is no evidence to indicate that the action of B. F. is in any essential different from the action of O. T.

I have not the space to discuss the nature of the tuberculin reaction. It must suffice to say that in its broad features it is a hypersensitive reaction similar to the hypersensitive reaction to other foreign proteins. If this be so it is an advantage to have the protein as pure as possible and free from admixture of other proteins. For this reason Jochmann prepared his albumose-free tuberculin, growing tubercle bacilli upon medium free from protein.

Much emphasis has been put upon the source of the tubercle bacilli from which the tuberculin is prepared. It has been generally known that different strains of tubercle bacilli produce widely varying tuberculins. The variation is in the strength alone, the character of their effects being invariably the same. So much has been claimed for difference in diagnostic and therapeutic effect between tuberculin from human and tubercul in from bovine tubercle bacilli that it is of the greatest importance to emphasize that this statement applies with equal justice to products from these two sources. Römer (135), after an extensive investigation of the effects of tuberculin from human, bovine, and fowl tubercle bacilli upon animals (guinea-pigs, cattle, chickens, and rabbits), infected with human, bovine, and fowl tubercle bacilli, concludes that there is no essential difference in the character of the effects the three produce. Indeed, human and bovine tuberculin are so identical in their action upon infected animals that we may neglect to ascertain their source. These results are fully sustained in a recent publication of Weber and Dieterlein (163). These authors tested the effect of human and bovine tuberculin upon tuberculous cattle and upon guinea-pigs infected with human and bovine bacilli. While they find that even marked differences in potency may exist in tuberculins from different sources, the quality of the reaction is always the same.

I hope that I have made it clear that the selection of a tuberculin is a very simple matter, since practically all tuberculins contain tuberculo-protein and are therefore efficient. I hope that I have also shown that all alleged proofs of the superiority of one tuberculin over another are specious. Indeed, the one conclusion that may justly be drawn from the foregoing exposition is that the simplest tuberculins are to be preferred

1 For a complete consideration see Hamman and Wolman (53).
if only for economy. Upon theoretical grounds Jochmann's A. T. has some advantages, and for this reason is becoming popular. In practice, however, these advantages are unimportant. Because they are the simplest we advise a choice to be made between O. T., B. F., A. T., T. R., and B. E. However, it may be possible that although these tuberculins are essentially equivalent, still there may be minor differences that make the selection of one or another of them more desirable. For instance, it is claimed that reactions come more unexpectedly and are more prolonged when bacillary emulsions are used than in treatment with the filtrates. The explanation for this difference may be purely mechanical, since it is difficult to get uniform suspensions of tubercle bacilli or coarse particles of their ground-up bodies. Many authors claim that patients displaying unusual sensitiveness to one preparation will tolerate another satisfactorily.

In speaking of the results of tuberculin treatment no doubt it was noticed that I disregarded entirely the particular tuberculin that had been employed. The results reported were obtained with different tuberculins. Those that have been most frequently mentioned in the various reports are Koch's O. T., T. R., and B. E.; Beraneck's tuberculin, Denys' B. F., Jochmann's protein-free tuberculin and the bovine tuberculins. In order to see whether in the treatment of any one form of tuberculosis better results were obtained with a particular variety of tuberculin I tabulated for each organ the choice tuberculin as it seemed to each author. I found that for all the organs the list is practically the same. For example, in the literature on the treatment of glands one of the following tuberculins is regarded by some authors as the most suitable for the treatment of glandular tuberculosis: Koch's O. T., T. R., or B. E., Beraneck's tuberculin, Denys' B. F., Jochmann's protein-free tuberculin. Now and again some other tuberculin is mentioned, but the three tuberculins of Koch, Denys, and Beraneck, with recently the protein-free preparations, are by far the most used. However, the individual preferences of authors may differ. Frequently mention is made, as by Bandelier and Roepke, or by Jochmann, that good results were obtained with any of the above tuberculins. We cannot, from a review of the literature, see that there is at present any clinical basis for preferring any one of the principal tuberculins over another. Preferences are often based on a worker's long-continued use of a special brand, and his consequent unwillingness to change. However, some writers feel that there is a demonstrable difference in the action of some of the chief tuberculins. For example, although Bandelier and Roepke think them all therapeutically efficient, they believe that O. T. causes more inflammatory changes at the focus, and that B. E. is more apt to give fever reactions than local changes. But they prefer B. E. as an antipyretic over O. T. when fever is already

1 See Wolff-Eisner (171) and Blumel (22).
present. Brown has also noticed fever reactions with B. E., unac-
accompanied by other symptoms. Kehl (73) thinks O. T. an efficient
antipyretic, while Neuman (114) prefers T. R. or B. E., as does F.
Krause. However, Denys' B. F. and Beranek's tuberculin have strong
defenders of their antipyretic action. Bandelier and Roepke think T. R.
or B. E. produce more antibacterial immunity than O. T., and yet Goetsch
had to change from T. R. to O. T. in order to cause the disappearance of
the bacilli from the sputum. Work with agglutinins does not bring us
any nearer to a reasonable choice, since the weight-relation of the various
brands has been so often disregarded. As for the protein-free prepara-
tions, Joehmann well says that, while they are somewhat less apt to cause
fever than the others, the therapeutic effect is about the same. In other
words, while the tuberculins grown on protein media contain small
amounts of non-specific pyrogenic substances, these are not enough to
hinder the therapy, and, furthermore, only infrequently is the fever due
to the non-specific, rather than to the specific, components.

SELECTION OF PATIENTS

The physician, assured of the value of tuberculin, and having chosen
the preparation he wishes to use, will next look about among his patients
for cases suitable for treatment.

Bearing upon the choice of patients, it is important to point out
again that tuberculin is not an antitoxin, not a neutralizer of the poisons
produced by the disease, nor a germicide directly killing the tubercle
bacillus. Whatever differences may exist between opinions regarding the
exact mode of action of tuberculin, all observers are agreed upon this
much, namely, that tuberculin acts by stimulating the patient, stimulating
him to elaborate protective substances, or to an inflammatory reaction
about the area of infection. In a sense tuberculin is a tax upon the
patient, a whip to his natural powers of protection. With this one point
firmly fixed in mind the common sense of any shrewd physician will
guide him in the choice of patients suitable for tuberculin treatment.

Patients with their reacting powers spent in a long fight with the
disease, or overwhelmed by a severe or widespread infection, will not be
benefited by tuberculin. We would more easily believe that the treatment
under such conditions is harmful. A patient in good general condition
with an extensive lesion is in better condition to profit by the treatment
than one with a small lesion that is producing constitutional symptoms and
progressive exhaustion. To apply this principle specifically we might
elaborate it as follows:

(1) The most suitable patients for treatment are those with small
localized lesions that are not producing constitutional symptoms, namely,
early pulmonary tuberculosis, tuberculosis of glands, bones, and so on. You will no doubt remark that it is a wise forethought to select for tuberculin treatment those patients who respond most readily to any form of treatment. But why should not tuberculin be most beneficial to those most easily benefited? It is in keeping with our estimate of tuberculin, not a cure, but a favorable factor. Besides, I hasten to add that, while tuberculin does most good to patients with circumscribed local lesions, its most striking effects are produced in patients with more extensive disease.

(2) The most striking results of tuberculin treatment are seen in patients in good, or, at least, fair, general condition, with moderately or far-advanced lesions. Many of these patients have reaped a measure of improvement from the hygienic-dietetic treatment, but have then for months remained stationary, going neither forward nor backward. Tuberculin is often just the stimulation they need to start them upon a course of rapid improvement. Such instances are not isolated; every one who has used tuberculin can point to a number of them, patients whose rapid and prolonged or lasting improvement has been one of the keenest satisfactions of his medical work.

(3) Entirely unsuitable for tuberculin treatment are patients exhausted by the disease or with an actively progressing infection. Advanced cases with fever and emaciation are to be excluded. Likewise instances of acute disseminated tuberculosis. I feel that one must look with suspicion upon reports of tuberculous meningitis cured by tuberculin treatment.

(4) Between the group of patients definitely suitable for tuberculin treatment and the group definitely unsuitable there is a large class of borderline cases. They are not hopelessly advanced, and still have symptoms that clinicians refer to as the symptoms of activity of the disease. No general rule can be laid down about such cases; some are certainly benefited by tuberculin, some apparently receive no benefit. When tuberculin is cautiously given it does no harm, and in many patients belonging to this borderline group it must be started tentatively with a readiness to discontinue or to push on according to the results obtained.

In my own experience I have not seen striking benefits from tuberculin administered to patients with fever. Many authors, e. g., Litzner (101), Engel (39), Krause (87), praise it extravagantly as an antipyretic, and I am willing to concede that my disappointment has been due in part to my work being largely with ambulant patients. When patients with fever fail to respond to prolonged rest in bed, in my experience they usually fail to respond to tuberculin. And in patients with fever, or with their nutrition below par, a preliminary course of rest and out-of-door treatment will pave the way for a more satisfactory tuberculin cure.

Our studies of tuberculin statistics, if they have not convinced us, have at least pointed definitely to the more lasting results in those treated
with tuberculin in comparison with those not so treated. Tuberculin treatment will therefore find a large field of usefulness in patients who have lost their symptoms of the infection under a hygienic-dietetic or sanatorium régime, but still display evident signs of the tuberculous lesion. Generally employed in such cases we believe it will improve the ultimate results of sanatorium treatment.

Many observers claim that the results of tuberculin treatment in surgical tuberculosis are far superior to those obtained in pulmonary tuberculosis. While literally true, relative conditions are not taken into account in this statement. I have emphasized the influence of the general condition of the patient upon tuberculin treatment. Surgical tuberculosis is usually unaccompanied by constitutional symptoms while such an association is the rule in pulmonary tuberculosis. Experience has convinced me that pulmonary tuberculosis is as promising a field for tuberculin treatment as other forms of the infection if the condition of the patient be considered.

**THE GENERAL PRINCIPLES OF TUBERCULIN TREATMENT**

The physician, having chosen the tuberculin preparation he will use, and having selected a number of suitable patients, must have further a specific plan of action before beginning the treatment. He must have in mind very clearly just what he wishes to do. With this purpose firmly fixed he can easily avoid the difficulties and uncertainties that beset him.

Although there are innumerable variations in the methods of administering tuberculin, still, in a general way, these methods may be reduced to two: (1) the method of giving small doses and repeating the same small dose at stated intervals; (2) the method of starting with small doses and progressively increasing the dose, varying the time interval, and rate of progression to suit individual conditions.

**Method of Continuous Minimal Dosage**

The method of continuous minimal dosage was devised by Wright (172 and 173), and has received its main support from him and his school. Wright's contentions are based entirely upon his views regarding phagocytosis. As is well known he has demonstrated that the blood serum normally possesses the property of preparing foreign material for the phagocytic action of leukocytes. The substance in the serum that gives it this property he names opsonin. He has devised an ingenious method for estimating the opsonic power of serum, the resultant being termed opsonic index. The opsonic index toward different bacteria is regarded as specific. It varies in different individuals under influences that are not altogether
understood. However, the main influencing factor is contact with the particular organism under consideration. When infection occurs the first movement of the opsonic index is downward (negative phase), followed, if the individual responds satisfactorily, by a rapid rise above the previous level (positive phase). In the fluctuations of the opsonic index Wright sees a valuable control of the response of the individual to the infection. Fluctuations similar to those occurring in natural infections may be brought about by the injection of vaccines prepared from the organisms. The variations of the opsonic index following such injections determine the size and interval of the dose.

These principles applied to a study of tuberculous infection led Wright to advocate for treatment small doses of T. R. given at intervals of from seven to ten days. The final test of the efficacy of a dose is the determination of the degree of opsonic response. But many such estimations have led to the adoption of a dose between 0.05 c. mm. and 0.001 c. mm. as generally applicable, and ten days as the best general interval.

Wright's work is to be welcomed as an attempt to put tuberculin treatment upon a sound experimental basis. However, the results of subsequent investigations have shown that the method of determining the opsonic index is far from accurate, and that the range of error is so wide that no legitimate inferences can be drawn from slight variations.\(^1\) Besides, we would scarcely be justified in using a single immunity reaction as a gauge of the total reaction to an infection. Such a conclusion would follow only if extensive investigation established a constant relation between the two, and no such relation has been established for the opsonic index in tuberculous disease. It is true that Wright regards opsonic power as a by-product of antibodies possessing other functions and therefore a convenient indication of the amount of general antibody formation in the body. However, this view is not firmly grounded.

Indeed our knowledge of the relation of so-called antibodies to the degree of immunity and the intensity and course of the infection is very meager. In many clinical discussions of tuberculosis the word "antibodies" is used so confidently and so promiscuously that one is led to believe that this charmed word contains closed within its ten brief symbols all that mortal ever has learned or ever can learn of the disease. It explains infection and resistance; when it is whispered the veil that has so long hung before the tuberculin reaction falls away; a little more or a little less decides why we have tuberculosis and how we get well of it. Briefly, in some circles, every question that may be put about the infection is satisfactorily answered by this mystic symbol. That it is a convenient term and has a genuine significance based upon experimental data is true, but it loses all sense and dignity when detached from this support, it is bantered about as the open sesame to the knowledge of infections.

\(^1\) See for instance Moss (111).
GENERAL PRINCIPLES OF TREATMENT

I have already spoken of the contradictory evidence pertaining to the occurrence of complement-absorbing bodies in the serum. Agglutinins and precipitins bear no constant relation to the course of the disease. As has been said, no antitoxin in the sense of a substance capable of neutralizing tuberculin has ever been demonstrated.

Römer (134) has applied the methods of demonstrating the various immune antibodies to the serum of his animals of proved strong resistance to reinfection, and has found none to correspond regularly with the degree of immunity. Agglutinins are almost constantly present, but may not exceed the amount present in normal animals. Immune animals may fail to show complement-absorbing antibodies, while the serum of others completely inhibits hemolysis. He was unable to demonstrate antitoxin in the sense of a substance capable of neutralizing tuberculin. The serum of immune sheep has no influence upon tubercle bacilli allowed to remain a long time in contact with it. It is not possible passively to transfer immunity through the serum from a tuberculous to a non-infected animal.

For a long time the method of giving small doses continuously drew support from considerations flowing out of our knowledge of anaphylaxis or hypersensitiveness. To make the matter clear we must go back to the original experiments of Koch (78). He tells in a very graphic way how he came to hit upon the use of tuberculin in treatment. "When one inoculates a healthy guinea-pig with a pure culture of tubercle bacilli the wound as a rule closes and in the first few days seems to heal. However, in from ten to fourteen days a hard nodule appears, which soon breaks down, leaving an ulcer that persists to the time of death of the animal. There is quite a different sequence of events when a tuberculous guinea-pig is inoculated. In tuberculous animals the inoculation wound likewise promptly unites. However, no nodule forms, but on the next or second day after a peculiar change occurs. The point of inoculation and the tissues about, over an area of from 0.1 to 1 cm. in diameter, grow hard and take on a dark discoloration. Observations on subsequent days make it more and more apparent that the altered skin is necrotic. It is finally cast off and a shallow ulceration remains which usually heals quickly and permanently without the neighboring lymph glands becoming infected."

Healthy animals, then, react in a very different way from tuberculous animals to inoculations of tubercle bacilli. Extending Koch's early experiments it has been shown that tuberculous animals react in one of three ways to inoculation of tubercle bacilli:

(1) If a large number of tubercle bacilli are injected the animal dies in a few hours with symptoms of a profound intoxication.

(2) If the dose be small there is a prompt reaction about the site of injection which destroys the tubercle bacilli and prevents infection even of the regional lymph glands.

(3) If the size of the dose be larger than that which the animal is
able to resist, but not large enough to liberate acute fatal intoxication, infection does occur, but the resulting lesions are chronic and slowly progressing as compared with those produced by the same dose in normal controls.

Therefore, animals with tuberculosis can resist successfully reinoculation of tubercle bacilli in quantities surely fatal for normal animals, although the same mechanism which protects under these conditions is destructive when the number of bacilli is very large. The acute death following large doses has been studied in detail by Bail (8), the immunity to small doses most thoroughly by Römer (136). These results, so contradictory at first sight, are easily reconcilable. It is reasonably probable that the mechanism, whatever it may be, which causes the immediate toxic reaction on reinfection is the same upon which the animal withstanding this reinfection depends for its complete protection. How analogous these phenomena are to the general principles of anaphylaxis is at once apparent. The animals have by one infection been rendered hypersensitive to subsequent contact. This hypersensitiveness is, as we have shown, a valuable protective asset, but if the reinfecting dose be large the animal succumbs with the symptoms of an acute intoxication.

V. Behring, Koch (81), and Heymans (60) have shown that calves may be protected against many times the fatal dose of bovine tubercle bacilli by injections of living human tubercle bacilli. Following this immunizing injection calves do not develop gross tuberculous lesions, but do acquire tuberculin hypersensitiveness; that is, they react to subcutaneous injections of tuberculin just as tuberculous animals do. After about a year tuberculin hypersensitiveness is lost, and as it dies out the animals again become susceptible to inoculation with bovine tubercle bacilli.

Resistance to tuberculous infection can be conferred artificially only by the introduction of living tubercle bacilli, and Trudeau (158) has shown that the more virulent the organism the greater the protection. According to Römer (135) tuberculin hypersensitiveness runs remarkably parallel with the intensity of experimental infections.

These results indicate that a close relation exists between protection against tuberculous infection and hypersensitiveness to tuberculo-protein, although we cannot say definitely that the hypersensitiveness and the protecting mechanism are the same. Indeed, Krause (88) and Austrian (4) have found that animals made hypersensitive by the injection of pure tuberculo-protein are more susceptible to infection than normal animals.

These experimental results have been applied to clinical conditions in man and emphasized chiefly by Römer (137), Hamburger (52), and Wolff-Eisner (10). They regard tuberculin hypersensitiveness, or the mechanism of which it is an expression, as a valuable asset in the fight
against tuberculous disease. The question has been discussed interestingly by Baldwin (10). Wolff-Eisner views with alarm any measures taken to diminish hypersensitiveness. As is well known, tuberculin hypersensitiveness is influenced in a limited way by tuberculin injections, rapidly increasing doses diminishing it, small, frequently repeated doses increasing it. Therefore he considers the latter method more desirable for treatment. As clinical evidence to support this view Hamburger points to the marked resistance that tuberculous individuals have to reinfection, and Wolff-Eisner attempts to establish a relation between the degree of hypersensitiveness and the severity of the infection. He ascribes important prognostic significance to the tuberculin reactions.

To summarize briefly the conclusions that seem justified from the work on tuberculin hypersensitiveness in relation to tuberculous infections: Animals with tuberculous disease have a strong resistance against reinfections with tubercle bacilli. They withstand many times the fatal dose, but when very large amounts are given they succumb with stormy symptoms of an acute intoxication.

Although the animals are highly resistant to reinfection, this reinfection does not localize or overcome the original infection. Unquestionably it modifies its course, but I wish to emphasize that the mechanism is protective, not curative.

The parallel course of this resistance to superinfection and tuberculin hypersensitiveness is so striking that we are inclined to attribute both phenomena to the same mechanism.

I have frequently spoken of tuberculin hypersensitiveness as hypersensitiveness to tuberculo-protein. This is true in a general way, but all of the characteristics of the tuberculin reaction have not been reproduced experimentally with pure tuberculo-protein. Perhaps the difference is quantitative, not qualitative. Immunity is conferred only by inoculation of living tubercle bacilli, and immunity and the development of all the characteristics of tuberculin hypersensitiveness (e. g., cutaneous hypersensitiveness) seem to depend upon tubercle formation, at least as far as we know they fail to occur unless tuberculous tissue is formed.

In spite of the close relation between tuberculin hypersensitiveness and resistance to reinfection Römer is unwilling to identify the tuberculin reaction with the hypersensitive reaction following reinoculation. The former may be absent in animals which show a marked reaction to new infection and, as he points out, animals acquire tuberculin hypersensitiveness following the injection of dead tubercle bacilli, though they develop no resistance against infection.

I have written at such length of the experimental work on hypersensitiveness because it has completely modified our views of infection and the course of the disease in man. Though the field is tempting I cannot

1 See Hamburger (52) and Baldwin (10).
enter it, and must hurry to the relation of hypersensitiveness to tuberculin treatment. What I wish especially to call attention to is the double-edged character of the weapon. It cuts in two ways, for while it protects against reinfection and modifies the course of the disease, it is likewise responsible for the constitutional symptoms that accompany the infection. Thus, if the infected organism be exhausted by overstimulation it pays too dearly for the protection. Vaughan has put this in a striking way when he speaks of the anaphylactic shock as death from over-protection. Even though death may not occur, wasting and the other symptoms of intoxication are as much phenomena of hypersensitiveness as the protection against reinfection. To persuade these hypersensitive phenomena to subside is the aim of rest and the other well-established principles of tuberculosis treatment, and unless the symptoms be severe, tuberculin in increasing doses is an important aid to this end. As tuberculin tolerance is acquired there follows usually a noteworthy change in the condition of the patient. The appetite and digestion improve, energy and vigor increase, and nervous symptoms abate. It is significant that with returning hypersensitiveness the usual symptoms of the disease again become prominent, to subside once more when tuberculin tolerance is re-established; that when relapse occurs hypersensitiveness reappears; and that as a general rule in manifest tuberculous disease, when it is impossible to overcome the patient's hypersensitiveness and procure even a moderate measure of tolerance for tuberculin, improvement in the general and local conditions does not occur.

I have so far been unable to confirm Wolff-Eisner's contention of the prognostic value of hypersensitiveness.¹ Our work with tuberculin in diagnosis and treatment has led us to believe that tuberculin hypersensitiveness in relation to tuberculous disease runs, roughly, somewhat as follows: Since nearly all adults are infected with tuberculosis, we assume a low grade of tuberculin hypersensitiveness to begin with. Should there be a fresh invasion of the body from within or from without the tuberculin hypersensitiveness rapidly rises. If the disease subsides and the individual recovers the hypersensitiveness gradually falls to a lower level; if the disease remains active the high level of hypersensitiveness persists and lasts until the body is overwhelmed and its resistance broken down completely by the disease when hypersensitiveness disappears. Therefore, while we allow that in rapidly advancing cases the absence of tuberculin hypersensitiveness is an ominous sign in early and moderately advanced cases, we consider a low grade of hypersensitiveness a more favorable indication than a high. The high level hypersensitiveness rebellious to tuberculin treatment we have found to be of particularly unfavorable prognostic import.

It is still an open question whether tuberculin immunity or the loss of

¹See Hamman and Wolman (53); Gellen and Hamman (45).
hypersensitiveness following the injection of increasing doses of tuberculin is identical with the loss of tuberculin reactivity that occurs in rapidly advancing tuberculous disease or during the course of other diseases, notably measles. The question cannot be answered until the fundamental mechanism of hypersensitiveness is better understood. My own impression from clinical observations is that the two cannot be the same. The loss of reactivity at the end of the disease is certainly an exhaustion phenomenon, while the loss following tuberculin treatment is certainly not due to exhaustion. The remarkable improvement in general condition so commonly accompanying tuberculin treatment makes such an explanation unreasonable. To Wolff-Eisner's contention that a high grade of tuberculin tolerance induced artificially will expose the patient to an acute exacerbation of the disease I may reply upon the experience of innumerable clinicians that the fear is groundless. True it is that tuberculin immunity cannot be identified with tuberculosis immunity. Tuberculous complications and relapses occur in patients with a very high degree of tuberculin tolerance, but they do not occur more frequently than they do in untreated, highly hypersensitive patients. Indeed, clinical experience indicates that they occur less frequently.

The final and most cogent argument against the method of administering small doses without progression is that the plan has found little favor with clinicians. Although largely tried it has been generally abandoned. All are on the outlook for experimental data that will guide us in tuberculin treatment. We recognize that our methods are empirical, but until experiments are more clearly pertinent clinical evidence must have its weight.

**Method of Increasing Dosage**

The method of tuberculin treatment that is most widely adopted and has behind it the force of accumulated clinical experience is the method of increasing dosage. It is true that there is a wide difference of opinion upon the details of the treatment, but the principles are fairly uniform.

There are two ways in which tuberculin may have a beneficial effect:

1. By stimulation or modification of the machinery of immunization, thus rendering the individual more resistant to the effect of the infection and aiding to limit the activity of the tubercle bacillus.

2. By direct stimulation of the focus of infection, thus promoting healing and, through the inflammatory reaction occasioned about the focus, bathing it more lavishly with the products of immunization.

I have already considered in some detail the first of these effects. Experimental evidence in regard to the relation of immunity reactions to infection and the progress of the disease is inconclusive. Agglutinins, precipitins, and opsonins are formed, but their rôle is not clear. About
hypersensitiveness and its significance we are far better informed. But
many details await further investigation. However, although we cannot
fully explain its mode of action, still it cannot be doubted that tuberculin
has a profound effect upon the condition of the patient. Its effect upon the
symptoms spoken of as toxic I have repeatedly indicated, and indeed this
effect is clinically so striking that naturally enough clinicians looked upon
tuberculin as a primary toxin and tuberculin treatment as antitoxin
stimulation. I have pointed out that this view is no longer tenable, but
the observations upon which the view was based are too firmly established
to be disregarded. To these observations we owe such current terms as
tuberculin immunity (Trudeau) and giftfestigkeit (Sahli). Indeed,
many experienced observers, notably Sahli and Denys, see in this so-
called antitoxic effect the full value of tuberculin treatment.

It will be remembered that Koch considered the tuberculin reaction
a necessary part of tuberculin treatment, feeling that the full effects of
treatment were not obtained unless reactions occurred. In later publica-
tions he has never completely relinquished the idea of their importance.
It is needless to review the experience of the first tuberculin era which
was guided by this concept. There is no one point of tuberculin treat-
ment upon which there is such general accord as the harmfulness of
severe and, particularly, repeated severe general reactions. After re-
peated reactions patients almost invariably have a prolonged and tedious
convalescence.

Although there is this general condemnation of severe reactions, still
in milder form their effects may be beneficial. When tuberculous lesions
are situated externally and are thus accessible to inspection slight focal
reactions are often observed unaccompanied by constitutional symptoms.
The view is rapidly gaining ground that such gentle stimulation fre-
quently repeated encourages healing. No doubt these mild focal reactions
constantly occur during tuberculin treatment. We do not know all the
details of the relation between focal reactions and constitutional symp-
toms, but evidence points to a close relation. Indeed, many authors
regard the symptoms of a tuberculin reaction as secondary to and de-
pendent upon the focal reaction.

I must allow that we can draw no sharp line between the mild focal
stimulation that we look upon as beneficial and the severe reactions that
we regard with alarm. Every one who has had experience with tuberculin
has seen occasionally marked improvement follow so directly upon a
tuberculin reaction that he has been forced to ascribe a beneficial in-
fluence to it. I have already commented upon the favorable effect upon
some individuals of Koch's violent method. Again, some patients improve
markedly in spite of, and I believe on account of, repeated mild constitu-
tional reactions.

I have said that there is a wide difference of opinion about the details
of conducting tuberculin treatment according to the method of slowly progressing dosage. However, for purposes of discussion it is convenient to divide the difference into two groups, accepting as the type of each the extreme opinions, while stating that most observers take an intermediate position.

The first group is represented by Löwenstein (5), Petruschky (6), Bauer and Engel (14), and others. We refer particularly to Löwenstein's method because it is embodied in the directions for use accompanying Hoechst's tuberculin, and is therefore widely circulated. The object of his plan is to reach high doses of tuberculin in the shortest possible time. Minor details of treatment are held subservient to this prime object. He begins by giving diagnostic doses of tuberculin to find to what amount the patient will give a general reaction. This initial dose having been determined, after a rest of from ten to fourteen days, treatment proper is begun with its repetition, or even with a dose a little higher. From this point on the dose is progressively and rapidly raised. If reactions occur the dose is repeated if necessary three or four times, and then again increased. Slight reactions are not held to be contraindications for enlarging the amounts. Above all, the dose must never be decreased for fear of stimulating hypersensitiveness and making further advance impossible.

The second group is represented notably by Trudeau (157), Sahli (8), and Denys (3). Theirs is a much milder procedure than the one advocated by Löwenstein. While the aim is to arrive at as high a grade of tuberculin tolerance as possible, the reaching of high doses is not the ultimate object. Each patient is carried as high as his own individual tolerance will permit, and is never forced onward through reactions. Treatment is begun with doses so small that no reaction will be produced, and then cautiously raised, the slightest evidence of approaching sensitiveness being watched for. When these occur the amount of tuberculin is reduced, or at least held at the same level, until the indications have completely disappeared. The essential feature of the plan then is to avoid the slightest reaction, and instead of attempting to reach an absolute high dose of tuberculin, to carry each patient to the measure of his individual tolerance.

It is at once apparent that which method we accept will depend entirely upon our attitude toward reactions. I am becoming more and more convinced that focal stimulation is the most potent factor in tuberculin treatment, but I am equally convinced that general reactions are often harmful. The contention of Sahli and other adherents of the gentle method of procedure is not that mild reactions do harm, but that, having no means of controlling their extent, there is constant danger of their surging out of bounds if we set about purposely to produce them. He feels that our first duty is to do no harm. I agree with Sahli that we succeed in reaching as high doses by the mild as by the more daring plan, that improvement is equally satisfactory and that less danger is run.
On several occasions I have abandoned this conservative plan and used tuberculin more vigorously, but each attempt was followed by numerous general reactions. My experience has been gained almost entirely upon ambulant patients. It is possible that under institutional care and supervision a more rapid increase in dosage can be successfully followed.

The keynote, then, to tuberculin treatment is to hit the happy medium between sufficient and not too much focal stimulation. If we are to err it is safer to err on the side of too little than on the side of too much, but too timid a procedure will not give the full benefit of tuberculin, whereas an occasional mild constitutional reaction will do no harm. We believe that by careful observation one can give the proper amount of tuberculin and at the same time avoid objectionable reactions.

Finally I must add that some authors contend that we must divide clinical tuberculosis into two groups, one group comprising mainly pulmonary tuberculosis, the other surgical forms of tuberculosis, and that we must use different methods of treatment in each. These authors believe that in surgical tuberculosis better results are obtained by the method of continuous small dosage, while in pulmonary tuberculosis gradual advance in dosage is to be preferred. The selection seems to hinge upon the question of constitutional symptoms; when these are present, as they usually are in pulmonary tuberculosis, tuberculin immunity or *giftestigkeit* is to be aimed at; when they are absent, as they usually are, in surgical forms, hypersensitiveness or immunization without tolerance is to be sought. Baldwin suggested years ago that such a separation might be useful, and the suggestion has been elaborated practically by Riviere and Morland (7). We may explain the subject best by the divisions that Riviere and Morland make.

In their book on tuberculin treatment these authors divide tuberculous infections into two groups, autotoxic tuberculosis and localized tuberculosis. We quote from them as follows:

If we glance at the disease tuberculosis for which these methods find employment we shall see a rational explanation of the success which both methods have achieved in a different class of cases. Tuberculin is naturally divisible into two well-recognized groups; on the one side stand the cases where it is still a local disease; on the other side those where the disease has spread so far into vascular organs that large unequal doses of autotuberculin enter the blood stream. It is obvious that such different conditions call for somewhat different treatment: in all cases an immunizing response can be obtained with advantage by the employment of tuberculin. But where auto-inoculation occurs this will interfere materially with treatment by small doses at long intervals, and no certainty in the response can be expected. For this reason, if for no other, it is convenient in such cases to obtain the necessary response from doses so large that the production of autotuberculin does not interfere. But another reason also exists for the establishment of tolerance in these cases, for it is owing to a want of this compensatory mechanism that such patients go down hill under the influence of doses of unequal size and spacing issuing from their areas of disease. In producing
tolerance to injected tuberculin there arises at the same time tolerance to auto-
tuberculin; auto-inoculations cease to produce the train of symptoms attributable
to the tuberculotoxin and the patient loses his fever, night sweats, malaise. This
amelioration of symptoms has been noted by all who have worked with the larger
doses of tuberculin. In cases where no auto-inoculation occurs, as in local disease,
there is no reason for giving tuberculin after this method. Thus the two methods
of tuberculin administration find their proper field in the treatment of the two
varieties of tuberculosis: the method of small equal doses at long intervals is
suitable for all cases of localized disease, but where auto-inoculation adds tuber-
culotoxin from other sources tolerance must accompany immunization and the
treatment by rising doses at short intervals is indicated. Thus the realm of the
method of Wright is the localized and surface tuberculosis so commonly found
in children: the realm of the method of Koch is par excellence the common tuber-
culosis of adult life, phthisis pulmonalis.

The argument is attractive and sounds plausible, but I am unwilling to
accept the evidence upon which it is based. Stripped of all useless theory,
the division of tuberculous infection into autotoxic and localized is based
solely upon clinical symptoms, for these are the only evidence that we have
of auto-inoculation. In this respect it so happens that pulmonary tuber-
culos is more commonly autotoxic than surgical tuberculosis, but there is
no essential difference between the two types of infection, for pulmonary
tuberculosis may be, in this sense, a localized infection, and surgical
tuberculosis may be autotoxic. To base tuberculin treatment upon symp-
tomatic variation would entail constant change in the plan of treatment.
Besides, in tuberculin treatment our object is not to induce tuberculin
immunity alone; we wish at the same time to produce some effect upon
the tuberculous lesion. As a matter of experience the two run hand in
hand, and whether one aims to reach tuberculin tolerance or to obtain the
maximal stimulation possible without general reaction, it will in nearly all
instances be necessary to gradually and progressively raise the dose. In
pulmonary or autotoxic tuberculosis we are eager to reach tuberculin tol-
erance as quickly as possible, but to reach this goal we must keep a wary
eye upon focal reactions and accompanying general reactions. Indeed,
this is a clearer and safer guide to dosage than to rely upon evidences
of decreasing auto-inoculation, because such evidence is difficult to ap-
preciate and is only a gross indicator. In localized tuberculosis we em-
phasize chiefly the focal stimulation, the production of tuberculin tolerance
being an unimportant accompaniment. However, I believe it is impos-
sible to get the desired focal stimulation without raising the dose.

The basis for the separation that Riviere and Morland make is their
statement (p. 15) that the method of producing tolerance is followed by
excellent results in pulmonary tuberculosis, but fails in localized tuber-
culos is, while small doses at infrequent intervals give excellent results in
localized tuberculosis, but fail utterly in pulmonary infections. I believe
this statement to be unfounded, for as far as one can judge from published
accounts, the method of increasing dosage has produced results in localized tuberculosis as satisfactory as, if not better than, the method of continued small dosage. Except in England, where Wright’s influence is felt, the latter method has been generally abandoned. Much of Riviere and Morland’s argument is expressed in terms of Wright’s work upon opsonins, terms which no longer carry conviction.

In the practical section dealing with the method of immunization without tolerance the authors discard opsonin determination and rely upon clinical observations to guide them. Their directions (p. 178) for finding the optimum dose would fit admirably into any scheme of tuberculosis treatment. One gets the impression that in the end the distinction is more academic than practical.

To put the conclusion of this important section briefly, the best method of using tuberculin in treatment is to give increasing doses with the purpose of producing the greatest amount of focal stimulation without liberating general reactions.

THE PREPARATION OF TUBERCULIN DILUTIONS AND METHODS OF ADMINISTRATION

Up to this point has been a long and tedious journey for the prospective tuberculin therapist, but at last he is ready to begin treatment. While he halts for a little rest we will take the opportunity to prepare the tuberculin dilutions.

For practical purposes we have found that the simplest method is to prepare a series of dilutions, each being one-tenth the volume strength of the former. Bottle No. 1 contains pure tuberculin; No. 2, 9 c. c. diluent and 1 c. c. tuberculin; No. 3, 9 c. c. diluent and 1 c. c. of 2; No. 4, 9 c. c. diluent and 1 c. c. of No. 3, etc. The diluent is 0.8 per cent. salt solution with 0.25 per cent. carbolic acid. To administer 1 c. mm. we would give 0.1 c. c. of bottle No. 3; 5 c. mm., 0.5 c. c. of bottle No. 3, etc. It has been customary to designate the dose of tuberculin in grams and milligrams, while the dilutions are almost invariably made by liquid measurement. This makes a difference in the actual amount administered, but the error is small. However, to be consistent I have in this paper adopted the c. mm. as the measure of dosage. The dilutions are best made in wide-mouthed, glass-stoppered bottles. They should be kept in a cool, dark place when not in use. The salt solution must be prepared carefully with distilled water and pure sodium chlorid. Impurities may cause endless annoyance by producing a flocculent precipitate which may not appear until after twelve to twenty-four hours. If the pipettes are sterilized there is no danger of contamination. Fresh dilutions should be prepared every two weeks. We have been unable to note change in strength in this period.
To make the dilutions one needs a flask for the sterile salt-carbolic solution, a number of wide-mouthed, preferably glass-stoppered, bottles, and two pipettes, one with relatively large bore, accommodating 10 c. c. of liquid and graduated in tenths of a c. c., one with finer bore accommodating 0.1 c. c. and graduated in hundredths of a c. c. The simplest method of procedure is as follows:

To one liter of distilled water add 8 gm. of pure sodium chlorid and 2.5 c. c. of pure carbolic acid. Dissolve, filter into a thin flask, and plug the mouth with absorbent cotton. The solution is best sterilized in an autoclave, but boiling for fifteen minutes on two consecutive days suffices. If sterilized by boiling 1,100 c. c. of water should be used to allow for evaporation. It is an advantage to distribute the liter of solution in ten small flasks, each containing 100 c. c., rather than to sterilize it in a large flask. Whenever the tuberculin dilutions are to be prepared a small flask of diluent is used and the remaining portion discarded so that the same flask is never used a second time, and danger of contamination is avoided. Seven bottles are sterilized by boiling, and numbered from two to eight, and the date noted upon the label. Into each bottle 9 c. c. of diluent is measured. To bottle 2, 1 c. c. of tuberculin is added and carefully shaken; to bottle 3, 1 c. c. of bottle 2, etc. If only the high dilutions are required it is economical to begin at bottle 3 by using 9.9 c. c. diluent and 0.1 c. c. of tuberculin, and to prepare the higher dilutions as above by adding to 9 c. c. diluent 1 c. c. of the contents of the next lower dilution.

The injections are made subcutaneously, so that when a local reaction occurs it can be readily detected. I have found the Record Syringe the most satisfactory of the many I have used. The injection may be made into any portion of the body, but the region of the back below the angle of the scapula is the desirable situation. Often the arm will be found more convenient, and one need not hesitate to make the injections there. Local reactions follow injections into the arm more readily than injections into the back, and if the reaction be extensive it is far more painful and incommodating upon the arm. The syringe and needle should, of course, be boiled before use, and care should be taken that the tuberculin dilutions remain sterile. The skin needs no other preparation than to be rubbed with alcohol.

Other routes of administration have been proposed. None of these have advantage over the subcutaneous, some are questionably effective, and all have decided disadvantages.

Intravenous injections were first made by Koch. They have been advocated by Rothschild (140) and Heermann (57). There is danger of severe reactions, and we are deprived of a valuable guide to dosage by the absence of the local reaction.

The oral route has been recommended by Freymuth (42). Tubercu-
lin (B. E.) in capsules for administration by mouth is marketed as phytosoremid. Krause (89) has also used it. The effects of tuberculin taken by mouth are uncertain. Huhs (64) believes it has no specific action.

Tuberculin has been administered by inhalation by Kapralik and v. Schroetter (70). The method is not adapted to general use.

Jacob (66) gave tuberculin intrabronchially through a catheter. No one has followed his method.

The cutaneous route has been advised by Poppelmann (129) and Wallerstein. If one wishes to give very small doses these can be injected subcutaneously and absorption thus be assured.

Applications of tuberculin directly to tuberculous lesions have been practiced by Senger, Crocker, Pernet, and Wolff-Eisner (10) in cutaneous lesions.

The Initial Dose of Tuberculin

Everything is now ready for treatment to be begun. What shall the first dose be? There are two methods of procedure:

1. To attempt to estimate the patient's tolerance for tuberculin and inject a dose just short of the one that will cause a reaction.

2. To select a dose that experience has taught to be safely below the reacting dose and rapidly to advance until symptoms of approaching intolerance supervene.

The first method depends for its success upon the accuracy of quantitative tuberculin tests. Ellermann and Erlandsen (37) have studied the question from the standpoint of diagnosis. They perform the cutaneous test by applying a drop of 32 per cent., 8 per cent., 2 per cent., and 0.5 per cent. old tuberculin upon the skin and making through each dilution a superficial incision 2 to 3 cm. long. After two minutes the tuberculin is mopped off with cotton and a protective dressing applied. To insure an uniform depth of incision they use a lancet with adjustable point. At the end of twenty-four and forty-eight hours the diameters of the resulting papules are carefully measured and the mean of the readings taken. From the mean difference between the papules (D) and the estimated diameter of the 4 per cent. papule (the mean of the four papules) they estimate the tuberculinintitre or grade of sensitiveness of the individual. Thus:

<table>
<thead>
<tr>
<th>Tuberculin, per cent.</th>
<th>24 Hours, mm.</th>
<th>48 Hours, mm.</th>
<th>Average, mm.</th>
<th>D</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
<td>6.5</td>
<td>7.2</td>
<td>6.9</td>
<td>2.1</td>
<td>P4 = 3.8 mm.</td>
</tr>
<tr>
<td>8</td>
<td>4.5</td>
<td>5.0</td>
<td>4.8</td>
<td>2.2</td>
<td>D = 2.0 mm.</td>
</tr>
<tr>
<td>2</td>
<td>2.2</td>
<td>3.0</td>
<td>2.6</td>
<td>1.8</td>
<td>T = 264 mm.</td>
</tr>
<tr>
<td>0.5</td>
<td>trace</td>
<td>1.0</td>
<td>0.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Ellermann and Erlandsen do not attempt to estimate from the cutaneous reaction the size of the dose which, injected subcutaneously, will liberate a general reaction. However, White (167) and his co-workers have sought to establish such a relation. They proceed as follows: The inner surface of the forearm is cleaned with alcohol and ether, and with the v. Pirquet scarifier an abrasion is made measuring 2 mm. in diameter, the base of which must show a bright pink color. Not the least drop of blood must be drawn, but it is essential that the pink color appear at the base. After having made the proper scarification a drop measuring exactly 0.01 c. c. of the tuberculin solution to be used is applied with a throttle pipette exactly over the point of scarification. This is then covered by a vaccine shield, kept in place by two strips of adhesive plaster, and the patient directed to hold the arm in a horizontal position for at least an hour, to prevent flowing of the drop. For the first test a 1 per cent. tuberculin dilution is used. If this gives no reaction after an interval of four days a second test is made with a stronger solution; if too violent a reaction occurs to 1 per cent. a weaker solution is employed for the second test. In this manner one searches for a solution to which the individual gives a minimal cutaneous reaction. A minimal cutaneous reaction is defined as a reaction that gives a redness and swelling that measures 4 to 6 mm. in diameter within seventy-two hours. When one is pressed for time two tests, with different strengths of tuberculin, always putting the weaker distal to the stronger, may be performed simultaneously. However, this procedure is inadvisable. White claims the greatest accuracy for this method, and states that from the minimal cutaneous reaction he is able to unerringly state the exact dose which, administered subcutaneously, will produce a local reaction and the exact amount necessary to liberate a general reaction.

The cutaneous test is at best a rough test, and our experience has led us to believe it does not lend itself to such a simple interpretation. Boardman (23) has repeated White's work with different results. He finds that cutaneous tests performed in the same way upon the same individual at the same time may vary as much as 5 mm. in diameter. Further, that the dilution is the important factor, the size of the drop within reasonable limits being negligible. Again, that absorption is complete at the end of ten minutes, and to leave the tuberculin in contact with the abraded skin longer does not increase the size of the reaction. Lastly, in the few patients tested the size of the dose which, given subcutaneously, liberated a general reaction did not bear a constant ratio to the dilution giving a minimal cutaneous reaction.

Mantoux (104), following the suggestion of Mendel, introduced the intracutaneous test. The test is performed by injecting from a sterile syringe 0.05 c. c. of a dilute solution of tuberculin through a fine needle, the point of which has been inserted into the skin. After
cleaning the skin of the forearm with alcohol it is drawn taut with the left hand held under the arm, and the needle introduced, with the aperture directed toward the outer surface of the skin. If the point of the needle is in the skin a white elevation occurs immediately upon the introduction of the solution, if in the subcutaneous tissue no infiltration is apparent. Mantoux employs a 1-10,000 dilution of tuberculin, thus injecting 0.005 c. mm. of tuberculin. We have found it convenient to inject dilutions of increasing strength. As the tuberculin is absorbed large amounts cannot be given without the risk of producing constitutional reactions. In performing the test we make four simultaneous injections. The first consists of 1-20 c. c. pure salt solution as a control; the second of 1-20 c. c. of a 1-1,000,000 dilution of old tuberculin, equals 0.00005 c. mm., the third of 1-20 c. c. of a 1 to 100,000 dilution of old tuberculin, equals 0.0005 c. mm.; the fourth of 1-20 c. c. of a 1-10,000 dilution of old tuberculin, equals 0.005 c. mm. If none of these areas react we may perform a second test upon the opposite arm, injecting 1-20 c. c. of a 1-1,000 dilution of old tuberculin, which equals 0.5 c. mm. In this way a more accurate estimate of the degree of hypersensitiveness is obtained than from a single injection. The test is very delicate, and satisfactory results can be obtained only by exercising extreme precaution. Five new syringes must be selected, and one marked for use with each dilution of tuberculin, and never be used for any other strength. In cleaning the syringes the wash water must not be ejected into the sterilizer. We have been able to obtain satisfactory results only by boiling the syringe used for making the control injection of sterile salt solution in a separate dish in which syringes used for tuberculin injections never come.

The reaction consists of infiltration and hyperemia about the site of injection analogous to the reaction to the cutaneous test. It appears in from six to eight hours, reaches its maximum in from twenty-four to forty-eight hours, and usually disappears in from six to ten days. The injection of sterile salt solution into the skin is followed by a definite traumatic reaction, indistinguishable from a mild tuberculin reaction. This reaction is at its maximum after twenty-four hours, and completely disappears in forty-eight hours. In order to use the salt solution as a control the tests must be read forty-eight hours after they are given.

Römer (138) has used the method with success as a quantitative test for tuberculin hypersensitiveness in animals. It has such evident technical advantages over the cutaneous test that it alone should be used for quantitative estimations. As yet there are no studies upon the relation of the intracutaneous reaction to the size of the subcutaneous dose that will liberate a general reaction. It is possible, and I believe probable, that no such constant relation exists.

Although one cannot estimate accurately from quantitative tests the optimal dose for beginning tuberculin treatment, still I believe the pro-
METHODS OF ADMINISTRATION

cedure will acquire more and more importance. Even now one can by its aid divide patients in respect to their hypersensitiveness into different groups. For instance, we might divide them into the highly sensitive, the moderately sensitive, and the weakly sensitive. To those in the last class we could at once give a larger dose than to those belonging to the first class.

The second method is entirely empirical. Experience with the various tuberculins has taught us the safe dose for each, that is, the dose that will produce no reaction. Having thus begun treatment at this point the dose is rapidly raised until reactions threaten. In the highly sensitive this point is reached early, in the weakly sensitive not until weeks or even months have passed.

Observers do not agree upon the exact size of the dose best suited to inaugurate treatment, but there is general uniformity of opinion. My experience has been mainly with B. F. and O. T. For B. F. I consider 0.0001 c. mm. the dose generally suitable for beginning treatment. For O. T. 0.001 c. mm. For T. R. and B. E. the initial dose is usually between 0.001 and 0.005 c. mm. It will be remembered that T. R. contains 10 mg. and B. E. 5 mg. of ground dried tubercle bacilli in each cubic centimeter. Some authors have considered it best to express the dose of these two preparations in terms of the tubercle bacillus content, but this method is very confusing. We have adopted the plan of expressing the dose of all tuberculins in terms of dilutions of the marketed product.

It will be seen that the initial dose of all tuberculins is somewhere in the neighborhood of 0.001 c. mm., and it is a satisfactory plan to adopt this amount as the initial dose of any tuberculin. Severe reactions never occur after this dose, and the mild reactions that sometimes follow can do no harm. Brown gives the smallest dose that in his experience caused a reaction as 0.0001 c. mm. B. F. As the accompanying illustration shows, I have seen a local and a slight general reaction in a child to 0.000,001 c. mm. B. F.:

<table>
<thead>
<tr>
<th>Date</th>
<th>Dose</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dec. 2</td>
<td>B. F.</td>
<td>0.000,01 c. mm.</td>
</tr>
<tr>
<td>Dec. 9</td>
<td>&quot;</td>
<td>0.000,001</td>
</tr>
<tr>
<td>Dec. 23</td>
<td>&quot;</td>
<td>0.000,000,5</td>
</tr>
<tr>
<td>Jan. 30</td>
<td>&quot;</td>
<td>0.000,001</td>
</tr>
</tbody>
</table>

W. L., white boy, aged 7 years. F. is well, M. has pulmonary tuberculosis. Child well until six weeks before coming to the hospital, when he complained of pain in the neck, and his mother discovered that the glands in the neck were enlarged. The child was in excellent general condition and examination showed no abnormality other than slight general glandular enlargement and marked enlargement of the cervical glands. The tonsils were enlarged and reddened. Tuberculin treatment was begun as follows:

1910.
TUBERCULIN TREATMENT

Jan. 3 B.F. 0.000,001 c.mm. Temp. to 99.6° F.; no local reaction; no constitutional symptoms.
Jan. 16 “ 0.000,002 “ No fever; slight local pain; no constitutional symptoms.

It was impossible to raise the dose quickly and on March 24, 1911, he was still getting 0.000,1 c.mm. The glands had decreased a great deal in size. Such an extreme grade of hypersensitiveness is very unusual.

SUBSEQUENT DOSES AND INTERVALS

The physician has administered the first dose of tuberculin. When shall the second be given, and upon what plan shall the dose be increased? The question of dose intervals has aroused a great deal of discussion. Many advanced arguments based upon experimental data to enforce their contention, but in the end we have accepted the verdict of empiricism and adopted the interval that practice has found most satisfactory.

Those who follow Wright select ten days as the best general interval. They conceive each tuberculin injection to be followed by a short negative phase, then a rapidly rising positive phase, and a slow return to the previous level. The full play of this immunity response, they think, requires ten days, and they do not inject a second dose until the effects of the first have worn off.

Pickert (182) advises an interval of from sixteen to twenty-eight days between doses, claiming that he finds the formation of antituberculin to reach its high point during that period. I have already spoken of the method used to demonstrate antituberculin, and have said that the results are inconclusive.

The empirical results of clinicians have made the selection of from three- to five-day intervals almost universal. Some observers hold to these doses throughout, others lengthen the interval when larger doses are reached. To be consistent a regular interval should be adopted, but in institutional work, and even in private practice, it is a great convenience to select two days of the week for tuberculin administration. That one dose is given at a three-day interval and the alternate dose at a four-day interval has, as far as we can judge, no effect upon the result of the treatment.

Our routine method at the Johns Hopkins Hospital is to administer the small doses twice a week until we have reached the level of the patient's tolerance, when we change to the week interval. If the patient shows no evidence of intolerance we change to the week interval when 10 c.mm. is reached.

In the section on the principles of tuberculin treatment I pointed out that our aim should be to get the greatest amount of focal stimulation without liberating general reactions. To apply this principle each patient
METHODS OF ADMINISTRATION

should be studied individually, and the signs that indicate an impending reaction carefully watched for. I am convinced that with care this balance may be satisfactorily maintained. Therefore, before speaking of an outline for raising the dose I must point out in detail the symptoms by which one may know that the limit of tolerance has been reached.

The Tuberculin Reaction

The symptoms of a tuberculin reaction may be divided conveniently into three groups: (1) the general constitutional symptoms; (2) the focal reaction or changes that occur about the diseased area; (3) the local reaction or changes that occur at the point of injection.

The constitutional symptoms are manifold and varied. They consist usually of a rise of temperature and pulse rate associated with one or more of the following symptoms: chilliness, general malaise, headache, general aching, pain in the joints, loss of appetite, nausea, and vomiting. After a severe reaction there is usually a loss of weight.

The focal reaction consists of inflammatory changes about the lesion. When the lesion is situated externally the reaction is easily appreciated, but when the focus is in an internal organ even severe reactions may go undetected. Koch's description of the reaction in lupus gives a good picture of the changes.

A few hours after the injection the diseased skin becomes red and swollen. As the temperature rises the swelling and redness increase and may reach such a marked degree that the tissue becomes brownish-red and necrotic. With the fall of temperature the swelling decreases and in a few days may completely disappear. The lupus areas are covered with crusts which dry and fall off, leaving, sometimes after a single injection, a smooth pink scar. It is remarkable how absolutely specific is the selection of tuberculin for tuberculous tissue; none of the surrounding skin or old scars shows the least evidence of reaction.

The symptoms associated with such a reaction depend upon the site of the lesion. For instance in pulmonary tuberculosis they are pain, increase of cough and expectoration, and changes in the previously observed physical signs; in tuberculosis of bone and joint increased redness, swelling, heat and pain, with more evident limitation of movement and the appearance or increase of crepitus; in tuberculosis of the genito-urinary organs pain, swelling, increased secretion, bleeding, increased frequency and pain on urination.

The local reaction consists of pain, soreness, redness, and swelling at the point where the tuberculin is injected.

In tuberculin treatment we wish to avoid tuberculin reactions, and therefore do not push the dose until these frank manifestations of a reaction occur. Nevertheless we look to these various manifestations in mild forms as the signal of approaching danger.
TUBERCULIN TREATMENT

Of the constitutional symptoms the most helpful guide is the temperature. It is the only phenomenon that we can accurately measure, and is the one that most commonly occurs as an isolated signal. For this reason we give it careful attention. Patients taking tuberculin should, with few exceptions, keep a daily record of their temperature. To facilitate such record keeping special forms have been devised. We have found a record book modeled after one used by Brown to be satisfactory. The accompanying sheet (page 353) is a specimen page. On the inside of the cover the following directions are printed:

INSTRUCTIONS

Now that you are to begin to take tuberculin it is important that you pay the greatest attention to keeping this record carefully and conscientiously. Whether we increase or decrease the amount of tuberculin you are receiving will depend entirely upon how you have stood the preceding dose, and the only way we can judge of this is from the record you keep. Your improvement depends, then, to a large extent upon the faithfulness with which you keep your record. Never put down a temperature unless you are sure of it, and never make any entry until you are sure that you understand the book.

Each page in this book will keep your record for a week.

As you see, there are seven columns. Put the date at the top of the column, and make a note after each symptom in the space immediately opposite it. You fill in each space every day, except the "tuberculin" space, which the doctor will fill in. After each symptom, if you have it, make a + mark. If you haven't it make an O. After "appetite," "digestion," "sleep," write "good" or "poor," as may suit the case. Under the heading "rest" write how many hours spent in bed, how many in resting in a chair. In filling in the number of hours spent in the open air include those spent in bed if you sleep on a porch or with your windows out. Under "diet" put down the number of pints of milk, the number of eggs and the number of tablespoonfuls of oil. If you have any symptom, no matter how trivial it may seem to you, which is not in this book, tell the doctor about it at your next visit.

Elevations even of a few fifths of a degree above the usual maximum temperature should receive careful consideration and their relation to the injection should be studied. As isolated phenomena they do not necessarily indicate a tuberculin reaction, but their presence should arouse our suspicion, and if other symptoms accompany the rise we must proceed more cautiously with the treatment. If the temperature has been constantly subnormal with wide daily variations in range, under treatment the mean level may rise gradually toward normal and the oscillations become smaller. Such an occurrence must be viewed as a favorable effect of the treatment.

As is well known, patients with tuberculous lesions, and particularly patients with pulmonary tuberculosis, seldom have a constantly uniform range of temperature. Besides the usual variations in the daily oscillations their temperature balance is easily disturbed by a variety of conditions. There is no feature of tuberculin treatment more difficult than to estimate
| DATE: | | | | |
| TEMPERATURE | 8 A.M. | | | |
| | 12 N. | | | |
| | 4 P.M. | | | |
| | 8 P.M. | | | |
| PULSE: | 8 A.M. | | | |
| | 12 N. | | | |
| | 4 P.M. | | | |
| | 8 P.M. | | | |
| WEIGHT: | | | | |
| TUBERCULIN: | Dose | | | |
| PLACE OF INJECTION: | Pain | | | |
| | Swelling | | | |
| | Enlarged Glands | | | |
| SYMPTOMS: | Appetite | | | |
| | Digestion | | | |
| | Nausea | | | |
| | Vomiting | | | |
| | Headache | | | |
| | Chilliness | | | |
| | Pain in Joints | | | |
| | Sleep | | | |
| | Nervousness | | | |
| STRENGTH: | As usual | | | |
| | Increased | | | |
| | Decreased | | | |
| COUGH: | As usual | | | |
| | Increased | | | |
| | Decreased | | | |
| SPUTUM: | As usual | | | |
| | Increased | | | |
| | Decreased | | | |
| | Blood in Sputum | | | |
| | Pain in Chest | | | |
| | Shortness of Breath | | | |
| REST: | In Bed | | | |
| | Sitting Down | | | |
| | Exercise | | | |
| | In Open Air | | | |
| DIET: | Milk | | | |
| | Eggs | | | |
| | Oil | | | |
| | Total Gain in Weight | | | |
justly the relation of such disturbances to tuberculin administration. Certain general features aid us. Most helpful of these is careful observation of the point of injection. As our experience grows we emphasize this association more and more. Febrile reactions to tuberculin seldom occur without an accompanying local reaction unless preceding injections have been followed by local reactions. Not uncommonly a number of injections are followed by soreness and swelling, then suddenly when the dose is raised or repeated a general reaction supervenes, although after this particular injection no local changes occur. Denys refuses to consider any febrile elevation coming on after forty-eight hours, due to the tuberculin injection. However, Brown believes it may be delayed for from forty-eight to sixty hours. I have never observed a reaction to tuberculin come later than thirty-six hours after the injection.

Temperature elevations occurring during tuberculin treatment, and not due to the injections, may be grouped in three classes: (1) Temperature elevations due to external influences, over-exertion, fright, emotions. An unexpected visit may produce a decided rise, as may an animated conversation or excitement, as over a game of cards. Sometimes it is not possible to ascribe the temperature elevation to any definite cause, as the following record illustrates:

Leon T., white male, age 23 years. Came to the hospital August 15, 1913, complaining of enlarged glands in the neck. The glands began to enlarge six weeks before. Examination showed a sparely nourished young man of healthy appearance. There were a few indefinite signs at the top of the right lung. The cervical glands on the left side of the neck were somewhat enlarged and there was one large fluctuating gland at the left angle of the lower jaw. The gland was aspirated and 4 c. c. of bloody purulent material withdrawn. There was a reaction to the 1 per cent. conjunctival test and the cutaneous test was strongly positive. Tuberculin treatment was begun on August 15 with 0.000,01 c. mm. B. F.

<table>
<thead>
<tr>
<th>Date</th>
<th>Concentration</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aug. 26</td>
<td>B. F. 0.000,03 c. mm.</td>
<td>No fever; slight local reaction; no constitutional symptoms.</td>
</tr>
<tr>
<td>Aug. 31</td>
<td>&quot; 0.000,04 &quot;</td>
<td>No fever; no local reaction. The skin over the gland has become very red, and, as spontaneous rupture is imminent, the gland is incised and drained.</td>
</tr>
<tr>
<td>Sept. 9</td>
<td>&quot; 0.000,05 &quot;</td>
<td>Immediately after the injection the temperature began to rise, and at 8 P. M. in the evening had reached 103° F. The following day the temperature was normal and the patient felt perfectly well. There was no local reaction and no focal reaction.</td>
</tr>
<tr>
<td>Sept. 13</td>
<td>&quot; 0.000,03 &quot;</td>
<td>No fever; no local reaction; no constitutional symptoms.</td>
</tr>
<tr>
<td>Sept. 16</td>
<td>&quot; 0.000,05 &quot;</td>
<td>No fever; no local reaction; no constitutional symptoms.</td>
</tr>
<tr>
<td>Sept. 19</td>
<td>&quot; 0.000,08 &quot;</td>
<td>No fever; no local reaction; no constitutional symptoms.</td>
</tr>
</tbody>
</table>
Sept. 23. B. F. 0.000,1 e. mm. No fever; no local reaction; no constitutional symptoms.
Sept. 26 " 0.000,3 " No fever; no local reaction; no constitutional symptoms.
Sept. 30 " 0.000,6 " No fever; no local reaction; no constitutional symptoms.
Oct. 2 " 0.000,9 " No fever; slight local swelling; no constitutional symptoms.
Oct. 9 " 0.001,5 " No fever; no local reaction; no constitutional symptoms.

The temperature elevation that occurred so unexpectedly on the ninth of September could not have been due to the tuberculin injection. It came on too quickly after the injection, was not associated with a local reaction, and subsequently, although the dose was rapidly raised, no reactions occurred. (2) All patients with tuberculosis are susceptible to variations in temperature that are not readily explained. Such temporary elevations are now interpreted as evidence of auto-inoculation. On account of changes, probably circulatory, about the lesion, absorption is suddenly increased and the patient has an endogenous tuberculin reaction. Indeed such reactions often present the characteristic ear-marks of a tuberculin reaction, and aside from the absence of the local changes are indistinguishable from it. To this mechanism is ascribed the fever following exertion. This conception is the foundation of Patterson's (183) method of treating tuberculosis by graded exercise. (3) Intercurrent infections are a fertile source of temperature elevation. The beginning of an attack of tonsillitis, of grip, or of any infection may cause alarm until the course of events decides the diagnosis. The charts on page 356 show such elevations of temperature, one due to tonsillitis, the other to secondary syphilis.

During a tuberculin reaction the pulse usually follows the temperature curve. Bandelier and Roepke regard an increase in the pulse rate as a solitary signal of great importance. I cannot confirm this observation, though I admit I have paid less heed to the pulse than to the temperature.

The other constitutional symptoms need not be regarded separately; they may be considered as a group under the head of intoxication. I use the term intoxication in a descriptive, not a literal sense. After tuberculin administered subcutaneously for diagnosis patients often complain of general indisposition and malaise, though there is no rise of temperature. Occasionally during tuberculin treatment similar symptoms occur. The condition is ill defined and cannot be described with precision, but the patient complains of not feeling so well as usual, of depression, of loss of appetite, of headache, and of nervousness. Symptoms indefinite enough, it is true, but worthy of consideration, and when combined with loss of weight, of great importance. Indeed, loss of weight as an isolated symptom is sometimes the first warning of intolerance. It is, however, more
valuable as a sign of overdosage late in treatment than as a protection against suddenly appearing reactions. I have found that tuberculin in-

tolerance to small doses manifested by symptoms of intoxication and without an accompanying local reaction occurs commonly at the beginning

of treatment. Patients displaying such reaction often have a little fever and other symptoms of intoxication before tuberculin is begun, and the in-
METHODS OF ADMINISTRATION

Injections simply aggravate these symptoms. Apparently these patients have too little resistance to respond to tuberculin injections with a frank local reaction. The accompanying record illustrates such a condition:

Edith F., white female, age 22 years. Came to the hospital January 26, 1912, complaining of cough and expectoration. Examination showed a fairly well-nourished woman with marked involvement of both lungs and tubercle bacilli in the sputum. Her temperature was constantly a little elevated. Tuberculin treatment was begun February 2, 1912, with 0.000,01 c.mm. B. F.

Feb. 6 B. F. 0.000,02 c.mm. Temp. to 99.6° F.; no local reaction; feels well.
Feb. 9 " 0.000,04 " Temp. to 100.2°; no local reaction; pain in the chest and general depression.
Feb. 13 " 0.000,02 " Temp. to 99.2°; slight local reaction; feels well.
Feb. 16 " 0.000,02 " Temp. to 100.2°; no local reaction; feels well.
Feb. 20 " 0.000,03 " Temp. to 100°; slight local pain; feels well.
Feb. 23 " 0.000,01 " Temp. to 101°; no local reaction; pain in the chest.
Mar. 1 " 0.000,01 " Temp. to 99.2°; no local reaction; feels well.
Mar. 5 " 0.000,02 " Temp. to 100°; slight local reaction; feels well.
Mar. 8 " 0.000,03 " Temp. to 99.2°; no local reaction; poor appetite.
Mar. 12 " 0.000,05 " Temp. to 100°; no local reaction; headache and depression.
Mar. 15 " 0.000,05 " Temp. to 98.8°; no local reaction; has not felt so well.
Mar. 19 " 0.000,07 " Temp. to 99.2°; slight local pain and redness; cough increased.
Mar. 22 " 0.000,09 " Temp. to 100°; no local reaction; feels well.
Mar. 26 " 0.000,1 " Temp. to 100°; no local reaction; cough and expectoration increased.
Apr. 19 " 0.000,01 " Temp. to 100°; no local reaction; feels well.
Apr. 30 " 0.000,01 " Temp. to 100°; no local reaction; feels well.
May 10 " 0.000,01 " Temp. to 99.6°; no local reaction; fairly well; has been losing a little weight.
May 14 " 0.000,02 " Temp. to 99.6°; no local reaction; is weak; otherwise feels well.
May 17 " 0.000,02 " Temp. to 99°; no local reaction; feels weak.
May 21 " 0.000,03 " Temp. to 99.2°; no local reaction; feeling better.
May 24 " 0.000,05 " Temp. to 99°; no local reaction; feels well.
May 28 " 0.000,07 " Temp. to 99.6°; slight local pain; feeling very well.
May 30 " 0.000,07 " Temp. to 101°; considerable local pain and swelling; does not feel so well.
June 7 " 0.000,07 " Temp. to 99.8°; no local reaction; headache after the injection.
June 11 " 0.000,09 " Temp. to 99.4°; no local reaction; feels very well.
June 14 " 0.000,1 " Temp. to 100°; no local reaction; feels very well.
TUBERCULIN TREATMENT

June 18  B. F.  0.000,1 e. mm. Temp. to 99.7° F.; no local reaction; feeling fairly well.
June 21    "  0.000,2 " Temp. to 101.2°; no local reaction; cough increased.
July 16    "  0.000,2 " Temp. to 99.7°; no local reaction; feels fairly well.
July 19    "  0.000,4 " Temp. to 99.7°; no local reaction; coughing a good deal and general prostration.
July 26    "  0.000,6 " Temp. to 99.8°; no local reaction; feeling better.

From this point on the patient improved considerably, the temperature seldom went above 99.4° F. and the dose was more rapidly increased. On February 18, 1913, she received 75 c. mm. However, the improvement was only temporary, for in March she again began to have considerable fever and tuberculin was stopped. The patient died in December, 1913. In this case it was very difficult to say during the early period of treatment to what extent the tuberculin was responsible for the patient's general symptoms. She had fever when tuberculin was not given and the rises bear no definite relation to the tuberculin injections. In such instances one is often at a loss to know whether or not tuberculin should be continued. When this doubt exists it is better to discontinue tuberculin rather than to push on in spite of the constitutional symptoms.

The focal reaction is of some value in guiding dosage when the lesion is situated externally. In my experience local or slight general reactions nearly always precede visible focal reactions, but in localized tuberculous lesions we have less fear of deleterious effects from general reactions than in pulmonary tuberculosis, and we may push on through local reactions until focal changes occur or a severe general reaction arrests our efforts. This is not good practice for routine cases, and should be used; if at all, under special conditions. As regards pulmonary tuberculosis I have never observed changes in the physical signs that could be interpreted as indubitable evidence of a focal reaction in the absence of constitutional symptoms. I say indubitable evidence because the question of the interpretation of pulmonary focal reactions is variously answered. For instance, Otten (115) is satisfied to draw such an important conclusion from slight changes in the percussion note. I have not attained such astonishing finesse. Nor am I willing to follow Roepke (133), who accepts changes in the character of the breath sounds as sufficient evidence. I regard the appearance of fresh râles as the only reliable mark of a pulmonary focal reaction.

Lastly, we come to a consideration of the local reaction which is the most valuable of the three in calling our attention to the proximity of the borderline of tolerance. In speaking of elevations of temperature I emphasized the importance of the local reaction as an aid in their interpretation, and said that general reactions practically never occur without local changes to preceding doses. Since we have paid special attention to the local reaction as a guide in treatment I have never missed this relation.
It is demonstrated sufficiently in the various illustrations I have chosen; for instance, in the two following:

Mamie F., No. 2162, white, female, aged 19 years. Came to the hospital November 9, 1907, complaining of cough and weakness. Examination showed a poorly nourished, pale girl, with evidences of marked involvement of both lungs. Tubercle bacilli were in the sputum. Tuberculin was begun January 3, 1908, with 0.0001 c. mm. O. T. The dose was rapidly raised, and on March 24 she received 0.01 c. mm. The course after this was as follows:

<table>
<thead>
<tr>
<th>Date</th>
<th>Dose</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mar. 31</td>
<td>O. T.</td>
<td>0.02 c. mm.</td>
</tr>
<tr>
<td>Apr. 3</td>
<td>0.03</td>
<td>Temp. to 99.8°F; pain in the chest.</td>
</tr>
<tr>
<td>Apr. 7</td>
<td>0.03</td>
<td>Temp. to 99°F; feels well.</td>
</tr>
<tr>
<td>Apr. 10</td>
<td>0.01</td>
<td>Temp. to 98.8°F; feels well.</td>
</tr>
<tr>
<td>Apr. 14</td>
<td>0.01</td>
<td>No fever; feels well.</td>
</tr>
<tr>
<td>Apr. 18</td>
<td>0.02</td>
<td>No fever; feels well.</td>
</tr>
<tr>
<td>Apr. 21</td>
<td>0.03</td>
<td>No fever; feels well.</td>
</tr>
<tr>
<td>Apr. 24</td>
<td>0.04</td>
<td>Temp. to 99.2°F; feels well.</td>
</tr>
<tr>
<td>May 1</td>
<td>0.03</td>
<td>No fever; feels well.</td>
</tr>
<tr>
<td>May 5</td>
<td>0.04</td>
<td>No fever; feels well.</td>
</tr>
<tr>
<td>May 8</td>
<td>0.05</td>
<td>Temp. to 99°F; pain in the chest; cough increased.</td>
</tr>
<tr>
<td>May 12</td>
<td>0.05</td>
<td>Temp. to 99.2°F; feels well.</td>
</tr>
<tr>
<td>May 22</td>
<td>0.04</td>
<td>Temp. to 102°F; cough increased; general constitutional symptoms.</td>
</tr>
<tr>
<td>June 5</td>
<td>0.02</td>
<td>No fever; feels well.</td>
</tr>
<tr>
<td>June 9</td>
<td>0.01</td>
<td>No fever; feels well; patient is losing weight.</td>
</tr>
<tr>
<td>June 28</td>
<td>0.005</td>
<td>No fever; feels well. From this point on tuberculin is again increased and on September 15, 1908, patient received 0.1 c. mm.</td>
</tr>
<tr>
<td>Sept. 18</td>
<td>0.2</td>
<td>Temp. to 98.8°F; feels well.</td>
</tr>
<tr>
<td>Sept. 22</td>
<td>0.2</td>
<td>No fever; feels well.</td>
</tr>
<tr>
<td>Oct. 6</td>
<td>0.2</td>
<td>No fever; feels well.</td>
</tr>
<tr>
<td>Oct. 9</td>
<td>0.3</td>
<td>No fever; feels well.</td>
</tr>
<tr>
<td>Oct. 13</td>
<td>0.4</td>
<td>No fever; feels well.</td>
</tr>
<tr>
<td>Oct. 20</td>
<td>0.5</td>
<td>No fever; feels well.</td>
</tr>
<tr>
<td>Oct. 23</td>
<td>0.6</td>
<td>No fever; feels well.</td>
</tr>
<tr>
<td>Oct. 27</td>
<td>0.8</td>
<td>No fever; feels well; marked pain and swelling at the point of injection.</td>
</tr>
<tr>
<td>Oct. 30</td>
<td>1</td>
<td>Temp. to 101.6°F; cough increased; marked constitutional symptoms.</td>
</tr>
<tr>
<td>Nov. 13</td>
<td>0.1</td>
<td>No fever; feels well.</td>
</tr>
<tr>
<td>Nov. 17</td>
<td>0.2</td>
<td>No fever; feels well.</td>
</tr>
<tr>
<td>Nov. 20</td>
<td>0.3</td>
<td>No fever; feels well.</td>
</tr>
<tr>
<td>Nov. 27</td>
<td>0.4</td>
<td>No fever; feels well.</td>
</tr>
<tr>
<td>Dec. 1</td>
<td>0.5</td>
<td>No fever; feels well.</td>
</tr>
<tr>
<td>Dec. 4</td>
<td>0.6</td>
<td>No fever; feels well.</td>
</tr>
<tr>
<td>Dec. 8</td>
<td>0.8</td>
<td>No fever; local redness and swelling; no constitutional symptoms.</td>
</tr>
<tr>
<td>Dec. 11</td>
<td>0.8</td>
<td>No fever; slight local tenderness; no constitutional symptoms.</td>
</tr>
<tr>
<td>Dec. 15</td>
<td>0.8</td>
<td>No fever; no local reaction; feels well.</td>
</tr>
<tr>
<td>Dec. 18</td>
<td>0.8</td>
<td>No fever; no local reaction; feels well.</td>
</tr>
<tr>
<td>Dec. 22</td>
<td>0.8</td>
<td>No fever; no local reaction; feels well.</td>
</tr>
</tbody>
</table>
Dec. 29 O. T. 1 c.mm. No fever; no local reaction.

1909.
Jan. 5 " 2 " No fever; no local reaction; feels well.
Jan. 8 " 2 " Temp. to 99° F.; no local reaction; feels well.
Jan. 12 " 2 " Temp. to 99°; marked local reaction.
Jan. 15 " 1 " Temp. to 99°; local reaction; no constitutional symptoms.
Jan. 19 " 1 " No fever; no local reaction; feels well.

Injections were continued with about the same results as above outlined. After months of treatment it was impossible to raise the dose much above 1 c.mm. The patient showed considerable improvement in her general condition and in the symptoms, but there was practically no change in the condition of the lungs. During the early part of the treatment, which was carried on in 1908, we had not learned to place the dependence we now do upon the local reactions. There is, therefore, little mention made in the records of the conditions at the point of injection. No doubt if the proper vigilance had been exercised it would have been found that the patient showed definite local reactions long before the dose was reached that liberated the general reaction. As is evident from the above record, we paid more attention to the temperature as a guide to dosage than we did to local reactions. Besides illustrating the importance of the local reaction as an indicator to an oncoming general reaction, the case illustrates the impossibility of raising the dose beyond a given amount, a condition which is frequently encountered in tuberculin treatment.

Milton P., No. 4939, white, male, aged 25 years. Came to the hospital September 25, 1909, complaining of pleurisy with effusion, which had been present for four years. Examination showed bilateral pleural effusion and peritoneal effusion. Patient gave a marked reaction to the cutaneous test. Tuberculin treatment was begun October 1, 1909, with 0.0001 c.mm. B. F. The dose was gradually raised and a slight general reaction occurred on October 12 after 0.0005 c.mm. B. F. On March 4 he received 0.7 c.mm. B. F.

Mar. 11 B. F. 0.9 c.mm. Temp. to 99.2° F.; no local reaction; feels well.
Mar. 15 " 1 " No fever; no local reaction; feels well.
Mar. 22 " 1.5 " Temp. to 99.7°; no local reaction; pain in the chest.
Mar. 29 " 1.5 " Temp. to 99.2°; no local reaction; feels well.
Apr. 5 " 2 " Temp. to 99.4°; slight local reaction; feels well.
Apr. 8 " 1.5 " Temp. to 99°; no local reaction; feels well.
Apr. 15 " 2 " Temp. to 100°; marked local reaction; feels well.
Apr. 22 " 1 " Temp. to 99.3°; slight local reaction; feels well.
May 3 " 1.5 " Temp. to 100°; marked local reaction; feels well.
May 6 " 0.7 " Temp. to 100°; marked local reaction; chilliness, fever, and general constitutional symptoms.

Local changes must be looked for carefully, and the site of the previous injection always inspected before the following dose is administered. Usually patients complain of a little tenderness when the reaction is slight, of severe pain and of swelling when intense. However, though they make no complaint, inspection may reveal more or less swelling and induration. When such local changes are observed we must proceed cautiously if we wish to avoid general reactions. If the dose be raised or the
size of the local reaction increase with succeeding injections of the same
dose general reactions are imminent.

I must point out that all regions of the body are not equally sensitive to
tuberculin. This interesting fact has been studied with the cutaneous
reaction, and applies equally to subcutaneous injections. Local reactions
occur much earlier when injections are made in the arm than when the
back is selected. For this reason we prefer to administer tuberculin in the
subcutaneous tissue of the back. From the importance attached to the
local reaction as a guide to tuberculin treatment it is evident why I have
emphasized that injections should be made subcutaneously and not deeply
into the muscles.

With a clear appreciation of the signals of approaching danger the
physician is in a position to push on with tuberculin treatment. The
initial dose has been administered and a bi-weekly interval decided upon.
His first duty is to avoid reactions, but it is scarcely less important to
carry the patient as quickly as possible to the point of his tolerance, the
point where tuberculin gives its best results. Thus the aim of treatment is
clear, though its application is individual. The benefits of tuberculin treat-
ment cannot be measured in terms of the quantity of tuberculin adminis-
tered, for a large dose to one patient has the effect of a smaller one to an-
other. Each appropriate dose has its own full value, and the benefits of
treatment are derived throughout the course and are not summed up in
the size of the final dose. Many patients who never get beyond a moderate
dose are as happily influenced as others going uninterrupted to large
amounts.

The fundamental secrets of tuberculin treatment are now revealed, and
perhaps it is superfluous to develop them further. However, experience
has suggested a number of interesting details in the application of the
principles, and it will be helpful to review them.

During the preliminary period of small dosage it is safe and, I think,
advisable to double the amount of each injection until symptoms warn
that the level of tolerance has been reached, or if these do not appear until
0.1 c. mm. is reached. It is indeed very arbitrary to select 0.1 c. mm. as
the dose beyond which we must proceed with greater caution, but experi-
ence has taught us that reactions occur more commonly to doses from 0.1
to 10 c. mm. than at any other level. It is the period that requires the
greatest vigilance, for when 10 c. mm. is passed progress from then on is
usually unobstructed. When 0.1 c. mm. is reached the dose may be in-
creased by tenths. This plan, however, has evident disadvantages, since
the increase from 0.1 to 0.2 c. mm. is a hundred per cent. increase, while
from 0.9 to 1 c. mm. is but one-eleventh per cent. increase. In support of
this objection I may say that when the plan is followed reactions are par-
ticularly apt to occur after the first large increase. To obviate this in-
equality the first and second jumps may be divided and the latter length-
ened. Thus we would give 0.1, 0.15, 0.25, 0.3, 0.4, 0.5, 0.7, 1.0, etc. This plan is simple, and in practice works well. To make the increase of dosage equal Brown has devised logarithmic scales. He writes:

It is intended merely as a suggestion in controlling the dosage, which for each patient varies greatly, according to individual susceptibility, and is of use in giving any tuberculin, for all tuberculins are either in solution or suspensions in fluids. This scheme computed by Pope is based on a logarithmic scale, and is so arranged that in going from 0.1 to 1 c. c. of any solution two to twelve doses may be employed, while the rate of increase of dose is always constant. The average patient, in the writer’s experience, can take the sixth scale (six doses to each solution) without any danger of reaction, but some must go more slowly and a few, especially during a second course, may go more rapidly.

**Doses (Logarithmic Scale)**

<table>
<thead>
<tr>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3.2</td>
<td>2.2</td>
<td>1.8</td>
<td>1.6</td>
<td>1.5</td>
<td>1.4</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>10</td>
<td>4.7</td>
<td>3.2</td>
<td>2.5</td>
<td>2.2</td>
<td>2.0</td>
<td>1.8</td>
<td>1.7</td>
<td>1.6</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>10</td>
<td>5.6</td>
<td>4.0</td>
<td>3.2</td>
<td>2.7</td>
<td>2.4</td>
<td>2.2</td>
<td>2.0</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>10</td>
<td>6.3</td>
<td>4.7</td>
<td>3.7</td>
<td>3.2</td>
<td>2.8</td>
<td>2.5</td>
<td>2.3</td>
<td>2.2</td>
<td>2.2</td>
<td>2.2</td>
</tr>
<tr>
<td>10</td>
<td>6.8</td>
<td>5.2</td>
<td>4.2</td>
<td>3.6</td>
<td>3.2</td>
<td>2.9</td>
<td>2.6</td>
<td>2.6</td>
<td>2.6</td>
<td>2.6</td>
</tr>
<tr>
<td>10</td>
<td>7.2</td>
<td>5.6</td>
<td>4.7</td>
<td>4.0</td>
<td>3.5</td>
<td>3.2</td>
<td>3.2</td>
<td>3.2</td>
<td>3.2</td>
<td>3.2</td>
</tr>
<tr>
<td>10</td>
<td>7.5</td>
<td>6.0</td>
<td>5.0</td>
<td>4.3</td>
<td>3.8</td>
<td>3.8</td>
<td>3.8</td>
<td>3.8</td>
<td>3.8</td>
<td>3.8</td>
</tr>
<tr>
<td>10</td>
<td>7.7</td>
<td>6.3</td>
<td>5.3</td>
<td>4.7</td>
<td>4.7</td>
<td>4.7</td>
<td>4.7</td>
<td>4.7</td>
<td>4.7</td>
<td>4.7</td>
</tr>
<tr>
<td>10</td>
<td>8.0</td>
<td>6.6</td>
<td>5.6</td>
<td>5.6</td>
<td>5.6</td>
<td>5.6</td>
<td>5.6</td>
<td>5.6</td>
<td>5.6</td>
<td>5.6</td>
</tr>
<tr>
<td>10</td>
<td>8.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

If at any time during the course of treatment symptoms of reaction appear progress thereafter must be very cautious. As I have stated, local changes are usually the first evidence of approaching intolerance. At times the local reaction increases with each succeeding injection, even though the dose be not raised; again, it may decrease with later injections and the period of threatening intolerance be quickly passed. If the dose has been rapidly raised a constitutional reaction may occur with the first local reaction.

Following this plan, it is often possible to raise the dose uninterrupted until large amounts are reached. The following example is illustrative:

Edwina M., No. 9016, colored woman, aged 24 years. Came to the hospital August 9, 1912, complaining of cough. Examination showed an emaciated colored woman with impairment and harsh breathing over the right upper lobe. No tubercle bacilli found in the sputum. Tuberculin was begun on September 24, 1912, with 0.000,01 c. mm. B. F. and the dose rapidly raised as follows:

Sept. 27 B. F. 0.000,03 c. mm. No reaction.
Oct. 1 " 0.000,05 " No reaction.
METHODS OF ADMINISTRATION

Oct. 8 B.F. 0.000,09 c.mm. No reaction.
Oct: 11 " 0.000,1 " No reaction.
Oct. 15 " 0.000,4 " No reaction.
Oct. 18 " 0.000,8 " No reaction.
Oct. 22 " 0.001 " No reaction.
Oct. 25 " 0.005 " No reaction.
Oct. 29 " 0.009 " No reaction.
Nov. 1 " 0.02 " No reaction.
Nov. 8 " 0.06 " No reaction.
Nov. 12 " 0.09 " No reaction.
Nov. 15 " 0.1 " No reaction.
Nov. 19 " 0.3 " No reaction.
Nov. 22 " 0.6 " No reaction.
Nov. 28 " 0.9 " No reaction.
Nov. 29 " 1 " No reaction; patient caught cold; temp. went to 100.3° F.; temperature subsided after a few days in bed; there was no local reaction.

Dec. 6 " 3 " No reaction.
Dec. 10 " 6 " No reaction.
Dec. 12 " 10 " No reaction.
Dec. 17 " 20 " No reaction.
Dec. 20 " 50 " No reaction.
Dec. 24 " 100 " No reaction.
Dec. 31 " 150 " No reaction.
Jan. 7 " 200 " No reaction.
Jan. 14 " 250 " No reaction.
Jan. 21 " 300 " No reaction.
Jan. 31 " 350 " No reaction.
Feb. 14 " 400 " No reaction.
Feb. 21 " 500 " Temp. to 99°; some local pain and swelling.
Mar. 14 " 500 " Local pain and swelling.
Mar. 28 " 500 " No reaction.
Apr. 11 " 550 " No reaction.
May 1 " 600 " No reaction.
May 23 " 650 " No reaction.
June 8 " 700 " No reaction.
June 22 " 800 " No reaction.

When treatment was begun patient's weight was 99 pounds; her weight increased and in March she weighed 109 1/2 pounds. From this point on her weight again decreased and in September, 1913, was 100 pounds. Tuberculin was discontinued on account of the loss of weight.

When symptoms of tuberculin reaction appear in the absence of a general reaction the further course will depend entirely upon the behavior of the patient. The behavior of patients at this point may be roughly grouped into four types, if you will remember that the dividing line between the groups is very elastic.

(1) In a number, by slowly and cautiously raising the dose, this early period of hypersensitiveness is soon overcome, and thereafter we can rapidly rise to higher doses. For example, the following cases:
Bertha W., No. 6952, white girl, aged 6 years. Came to the hospital February 14, 1911, complaining of cough. Examination showed a fairly well nourished child in good general condition with enlargement of the cervical lymph glands and a little impairment over the manubrium and a little impairment and harsh breathing at the top of the right lung. She had reacted violently to the cutaneous test. Tuberculin was begun March 14, 1911, with 0.000,01 c.mm.

Mar. 17 B. F. 0.000,01 c.mm. No fever; no local reaction; no constitutional symptoms.
Mar. 21 " 0.000,1 " No fever; no local pain; profuse night sweats.
Mar. 24 " 0.000,5 " No fever; marked local reaction; no constitutional symptoms.
Mar. 28 " 0.000,5 " No fever; marked local reaction; general urticarial eruption.
Apr. 4 " 0.000,5 " No fever; no local reaction; cough increased.
Apr. 7 " 0.000,5 " No fever; marked local reaction; herpes labialis.
Apr. 11 " 0.000,6 " No reaction.
Apr. 18 " 0.000,8 " No reaction.
Apr. 21 " 0.001 " No reaction.
Apr. 24 " 0.002 " No reaction.
Apr. 28 " 0.003 " No reaction.
May 2 " 0.005 " No reaction.
May 5 " 0.007 " No reaction.
May 9 " 0.01 " No reaction.

After this the dose was rapidly raised and on February 1, 1913, she received 1000 c.mm. During treatment the child’s weight rose from 45 to 62 pounds. The glands decreased in size and there were no further signs of pulmonary involvement. In May, 1913, the child passed uneventfully through an attack of measles.

Edna S., No. 8288, white female, aged 20 years. Came to the hospital on February 9, 1912, complaining of cough and pain in the chest. She dated her illness from an attack of typhoid fever she had had in June, 1910. She had had a cough ever since. Examination showed a poorly nourished girl with signs of involvement of the right upper lobe. Tubercle bacilli were present in the sputum.

Tuberculin treatment was begun on February 14, 1912, with 0.000,01 c.mm. B. F. The dose was progressively raised and on July 26, 1912, she received 0.1 c.mm.

July 30. B. F. 0.3 c.mm. Temp. to 99.4° F.; slight local pain and swelling; indigestion.
Aug. 2 " 0.5 " Temp. to 99.5°; no local reaction; pain in the chest.
Aug. 6 " 0.7 " No fever; local pain and swelling; feels well.
Aug. 9 " 0.9 " Temp. to 99.5°; marked local pain and swelling; increase of cough.
Aug. 13 " 0.7 " Temp. to 99.2°; slight local pain and swelling; feels well.
Aug. 16 " 0.9 " Temp. to 99.2°; no local reaction; feels well.
Aug. 20 " 1.5 " Temp. to 99.2°; no local reaction.
Aug. 23 " 1.5 " Temp. to 99.4°; no local reaction; not feeling quite so well.
Aug. 27 " 1 " Temp. to 99.2°; no local reaction; feels very well.
Aug. 30 " 3 " Temp. to 99.4°; no local reaction; feeling well.
### METHODS OF ADMINISTRATION

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Temperature</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sept. 3</td>
<td>B. F. 5 c. mm.</td>
<td>Temp. to 99.9°F; no local reaction; pain through the chest; shortness of breath; not feeling quite so well; tuberculin temporarily omitted.</td>
<td></td>
</tr>
<tr>
<td>Sept. 17</td>
<td>“ 5 ”</td>
<td>Temp. to 99.4°F; no local reaction.</td>
<td></td>
</tr>
<tr>
<td>Sept. 24</td>
<td>“ 7 ”</td>
<td>Temp. to 99.4°F; slight local pain and swelling.</td>
<td></td>
</tr>
<tr>
<td>Oct. 1</td>
<td>“ 7 ”</td>
<td>Temp. to 99.2°F; no local reaction.</td>
<td></td>
</tr>
<tr>
<td>Oct. 8</td>
<td>“ 9 ”</td>
<td>Temp. to 99.4°F; no local reaction; feeling well.</td>
<td></td>
</tr>
</tbody>
</table>

The dose was then gradually raised and on November 19, 1912, she received 3.5 c. mm. Patient then left the city to enter the sanatorium. There had been little change in the patient's condition during the period of treatment.

Catherine S., No. 9416, white female, aged 23 years. Came to the hospital December 13, 1912, complaining of cough which she had had over six months. Examination showed a sparely nourished girl with marked infiltration of both lungs and slight infiltration of the larynx and thickening of the cords without ulceration. Numerous tubercle bacilli in the sputum. Tuberculin treatment was begun on December 17, 1912, with 0.000,01 c. mm. B. F.

| Dec. 20 | B. F. 0.000,03 c. mm. | No fever; no local reaction; no constitutional symptoms. |
| Dec. 31 | “ 0.000,05 ” | No fever; no local reaction; pain in the side. |
| Jan. 3 | “ 0.000,07 ” | No fever; no local reaction; no constitutional symptoms. |
| Jan. 7 | “ 0.000,09 ” | No fever; no local reaction; felt sick on the day of the injection. |
| Jan. 10 | “ 0.000,15 ” | No fever; no local reaction; no constitutional symptoms. |
| Jan. 14 | “ 0.000,2 ” | No reaction. |
| Jan. 17 | “ 0.000,4 ” | No reaction. |
| Jan. 21 | “ 0.000,6 ” | Temp. to 99.6°F; local pain and swelling; no constitutional symptoms. |
| Jan. 24 | “ 0.000,7 ” | No fever; considerable local pain and swelling; no constitutional symptoms. |
| Jan. 28 | “ 0.000,7 ” | No fever; slight local swelling; no constitutional symptoms. |
| Jan. 31 | “ 0.000,9 ” | Temp. to 99°F; no local reaction; no constitutional symptoms. |
| Feb. 4 | “ 0.001,5 ” | Temp. to 99.4°F; slight local pain and swelling. |
| Feb. 7 | “ 0.003 ” | Temp. to 100°F; no local reaction. |
| Feb. 11 | “ 0.005 ” | Temp. to 99.4°F; slight local pain. |
| Feb. 14 | “ 0.008 ” | No fever; slight local pain and swelling. |
| Feb. 18 | “ 0.015 ” | No fever; slight local swelling. |
| Feb. 21 | “ 0.015 ” | Temp. to 99.2°F; no local reaction. |
| Feb. 25 | “ 0.02 ” | No fever; local pain and swelling. |
| Feb. 28 | “ 0.02 ” | Temp. to 99°F; no local reaction. |
| Mar. 4 | “ 0.04 ” | Temp. to 99.2°F; no local reaction. |
| Mar. 7 | “ 0.07 ” | No fever; no local reaction. |
| Mar. 11 | “ 0.09 ” | No fever; no local reaction. |
| Mar. 14 | “ 0.1 ” | No fever; no local reaction. |
| Mar. 18 | “ 0.3 ” | Temp. to 100°F; no local reaction. |
| Mar. 25 | “ 0.3 ” | Temp. to 99.2°F; slight local tenderness. |
TUBERCULIN TREATMENT

Mar. 28 B. F. 0.4 c. mm. Temp. to 99° F.; no local reaction.
Apr. 1 " 0.5 " Temp. to 99°; slight local pain.
Apr. 4 " 0.7 " Temp. to 99°; no local reaction.
Apr. 8 " 0.9 " Temp. to 99.2°; slight local pain.
Apr. 11 " 1 " Temp. to 102°; no local reaction.
Apr. 19 " 0.5 " Temp. 99.2°; local soreness for two days.
Apr. 22 " 0.5 " Temp. to 98.8°; no local reaction.
Apr. 25 " 0.7 " Temp. to 99°; no local reaction.
May 2 " 0.9 " No fever; slight local swelling.
May 6 " 1 " No fever; no local reaction.
May 9 " 3 " No fever; no local reaction.
May 16 " 5 " No fever; some local pain and swelling.
May 23 " 5 " Temp. to 99°; no local reaction.
June 3 " 5 " Temp. to 99.2°; no fever; no local reaction.

From this point on the dose was rapidly raised in spite of slight local reactions. On November 4, 1913, she received 200 c. mm. B. F. During the course of treatment the patient's cough improved and the amount of expectoration decreased, but there was practically no change in general condition or in condition of the lungs.

(2) In a number of cases the patient's sensitiveness remains at a remarkably constant level, so that any effort to go beyond a certain dose is invariably followed by a general reaction. Such instances are not isolated, and a constant level hypersensitiveness may persist for years. The following cases are illustrative:

H. A. T., male, aged 40 years. History of intestinal disturbances extending over ten years. Operation in September, 1910, undertaken for what was thought to be chronic appendicitis, revealed marked fibroid tuberculosis of the cecum. After operation there was continued recurrence of symptoms suggesting temporary intestinal obstruction. Tuberculin begun August 24, 1911, with 0.001 c. mm. B. F. September 14 he received 0.01 c. mm.

Sept. 18 B. F. 0.02 c. mm. Temp. to 99.6° F.; definite local reaction; no constitutional symptoms.
Sept. 25 " 0.02 " No fever; little local soreness; no constitutional symptoms.
Oct. 2 " 0.02 " No fever; slight local reaction; no constitutional symptoms.
Oct. 9 " 0.03 " Temp. to 99.4° F.; slight local reaction; no constitutional symptoms.
Oct. 16 " 0.03 " Temp. to 99.2°; slight local reaction; no constitutional symptoms.
Oct. 23 " 0.03 " Temp. to 99°; no local reaction; no constitutional symptoms.
Oct. 30 " 0.03 " Temp. to 99°; no local reaction; no constitutional symptoms.

After this everything proceeds smoothly and on February 5, 1912, the patient received 0.3 c. mm. B. F. After this dose there was a local reaction, but no fever
and no constitutional symptoms. February 12 0.4 c. mm. was given, followed by a temperature elevation to 102.2°, marked constitutional symptoms and a focal reaction (pain and tenderness) in the abdomen. Tuberculin was discontinued and again resumed on November 5, 1912, with 0.001 c. mm. B. F. The dose was gradually raised and on March 4, 1913, he received 0.8 c. mm.

Mar. 11 B. F. 1.0 c. mm. No fever; no local reaction; no constitutional symptoms.
Mar. 18 2.0 Slight fever; slight local reaction; general malaise.
Mar. 25 1.0 No fever; no local reaction; no constitutional symptoms.
Apr. 1 1.5 Slight fever; local tenderness; general malaise.
Apr. 8 1.0 No fever; local tenderness; pain in the right iliac fossa for two days.
Apr. 19 0.5 No fever; no local reaction; slight tenderness in abdomen.
Apr. 26 0.5 No fever; no local tenderness; no constitutional symptoms.

The dose was then kept at about 0.7 c. mm. for a number of weeks. Tuberculin was discontinued from June 28 to August 13, when treatment was resumed with 0.1 c. mm.

Sept. 3 B. F. 0.6 c. mm. No fever; no local reaction; no constitutional symptoms.
Sept. 10 0.8 Moderate fever; marked local reaction; marked constitutional symptoms.
Oct. 30 0.1 No fever; no local reaction; no constitutional symptoms.
Nov. 8 0.2 No fever; no local reaction; no constitutional symptoms.
Nov. 15 0.3 No fever; local tenderness; no constitutional symptoms.
Dec. 3 0.4 No fever; no local reaction; no constitutional symptoms.
Dec. 10 0.5 No fever; local swelling and tenderness; no constitutional symptoms.
Dec. 20 0.5 No fever; slight local reaction; no constitutional symptoms.
Dec. 27 0.5 No fever; no local reaction; no constitutional symptoms.
Jan. 3 0.6 No fever; some local swelling; no constitutional symptoms.
Jan. 10 0.6 No fever; local swelling and soreness; no constitutional symptoms.
Jan. 17 0.7 Temp. to 100.6°; intense local swelling and soreness; extreme prostration.

Patient improved a great deal under treatment. Since tuberculin was begun he has had no further attacks of intestinal disturbance. His general condition has improved in a striking way. On August 16, 1911, his weight was 149 pounds; on November 18, 1913, it was 208 pounds, necessitating a restriction of diet.
Mary W., No. 2808, white, female, 39 years of age. Came to the hospital May 1, 1908, complaining of fever. She had had fever off and on for four years. Examination showed a thin, frail woman with slight thyroid enlargement and signs of infiltration in both upper lobes with râles. The sputum was negative. On May 1 she gave an intense general reaction to 5 c. mm. O. T. with signs of a focal reaction in the right upper lobe. Tuberculin treatment was begun June 26, 1908, with 0.000,01 c. mm. O. T. The dose was raised without encountering any difficulty and on December 22 she received 0.1 c. mm. O. T.

Dec. 26 O. T. 0.15 c. mm. Temp. to 98.6° F.; some local swelling; feels well.
Dec. 29 " 0.1 " Temp. to 98.6°; some local swelling; feels well.
Jan. 2 " 0.2 " Temp. to 99.2°; no local reaction; feels well.
Jan. 5 " 0.3 " Temp. to 99°; local swelling; feels well.
Jan. 8 " 0.1 " No fever; marked local reaction; feels well.
Jan. 12 " 0.1 " Temp. to 99°; slight local reaction; pains in the joints.
Jan. 15 " 0.09 " Temp. to 99.2°; no local reaction; pain in the side.
Jan. 22 " 0.1 " Temp. to 99°; slight local reaction; feels well.
Jan. 29 " 0.1 " Temp. to 99°; slight local reaction; feels well.
Feb. 2 " 0.1 " Temp. to 98.6°; slight local reaction; feels well.
Feb. 5 " 0.1 " No fever; very marked local reaction; feels well.
Feb. 9 " 0.1 " Temp. to 99°; no local reaction; feels well.
Feb. 12 " 0.1 " Temp. to 99°; no local reaction; feels well.
Feb. 16 " 0.1 " Temp. to 99°; no local reaction; pain in right shoulder.
Feb. 19 " 0.1 " Temp. rose to 100° several days after the injection and the patient had a great deal of pain in the right arm. The pain was due to swelling in the subdeltoid bursa, probably a tuberculous bursitis.
Mar. 5 " 0.05 " No fever; no local reaction; shoulder is much better.
Mar. 5 " 0.06 " Temp. to 98.6°; slight local reaction; shoulder still stiff and painful.

The end of March the patient left the city and treatment was therefore discontinued.

(3) There are patients who persistently remain at a given level, but under prolonged treatment gradually acquire a lower hypersensitiveness, and the doses may then be gradually increased. In our experience such a change in hypersensitiveness is usually associated with a marked improvement in the patient's condition. The following cases illustrate this point:

Ethel G., No. 7790, white girl, age 14 years, came to the hospital August 29, 1911. Referred from the Presbyterian Eye and Throat Hospital on account of a tuberculous keratitis. Examination showed a fairly well nourished child with some impairment and change in the breath sounds over both upper lobes. The corneal lesion was quiescent. The child reacted unusually severely to the cutaneous test, and during the reaction there was marked injection about the old corneal lesion suggesting a focal reaction. Tuberculin was begun September 19, 1911, with 0.000,1 c. mm. B. F. The dose was very slowly raised and on January 19, 1912, she received 0.01 c. mm. From this point on the course is very slow on account of repeated local reactions.
METHODS OF ADMINISTRATION

Jan. 23 B. F. 0.02 c. mm. Temp. to 99°F; no local reaction.
Jan. 26 " 0.03 " Temp. to 99°F; no local reaction.
Jan. 30 " 0.05 " Temp. to 99.2°F; some local pain; the eye was injected on the 31st.
Feb. 2 " 0.05 " No fever; no local reaction.
Feb. 6 " 0.06 " Temp. to 99.6°F; local redness and swelling.
Feb. 13 " 0.05 " No fever; no local reaction.
Feb. 16 " 0.05 " Temp. to 99.4°F; no local reaction.
Feb. 20 " 0.06 " Temp. to 99.2°F; no local reaction; sleeping poorly.
Feb. 23 " 0.08 " Temp. to 99.6°F; some local pain and swelling; has a cold.
Feb. 27 " 0.08 " Temp. to 99.6°F; some local pain and swelling.
Mar. 1 " 0.06 " Temp. to 99.4°F; no local swelling.
Mar. 5 " 0.08 " Temp. to 99°F; slight local pain and swelling.
Mar. 8 " 0.08 " Temp. to 99.8°F; marked local swelling.
Mar. 15 " 0.08 " Temp. to 99°F; slight local swelling.
Mar. 19 " 0.09 " Temp. to 98.6°F; no local reaction.
Mar. 22 " 0.1 " Temp. to 99.6°F; no local reaction; complains of having caught cold.
Mar. 26 " 0.1 " Temp. to 99.4°F; no local reaction.
Mar. 29 " 0.1 " Temp. to 99°F; no local reaction; feels very well.
Apr. 2 " 0.3 " Temp. to 99.8°F; no local reaction; eye has been inflamed.

On account of the difficulty experienced in raising the dose and the occurrence of what was apparently a focal reaction in the eye, tuberculin was discontinued temporarily.

Apr. 23 B. F. 0.1 c. mm. Temp. to 99°F; no local reaction.
Apr. 27 " 0.1 " Temp. to 99.4°F; no local reaction; feels well.
Apr. 30 " 0.1 " Temp. to 99.2°F; no local reaction.
May 3 " 0.2 " Temp. to 98°F; no local reaction.
May 7 " 0.3 " Temp. to 99.2°F; no local reaction.
May 10 " 0.5 " Temp. to 99°F; slight local reaction.
May 14 " 0.6 " Temp. to 99.8°F; no local reaction; feeling very well.
May 17 " 0.8 " Temp. to 98.8°F; no local reaction.
May 21 " 0.9 " Temp. to 98.8°F; slight local reaction.
May 24 " 1 " Temp. to 98.8°F; no local reaction.

From this point on the course went very smoothly and the dose was rapidly raised. On February 18, 1913, she received 500 c. mm. During treatment the patient's general condition and the eye improved.

(4) In a relatively small number of patients the measure of their tolerance is reached early, and either it is impossible to advance the dose without producing disagreeable symptoms or indeed in some further treatment increases the hypersensitiveness, and it is necessary to retreat to smaller doses or abandon tuberculin altogether. In our experience such patients rarely do well under any treatment.

The fourth group has received extended consideration under the caption of the supersensitive state. In this condition all efforts to push treat-
ment are without avail; indeed, our efforts but increase the intolerance. For instance, a patient may be started with a dose of 0.001 c. mm. and take increasing doses without apparent effect until 0.02 is reached, when a marked local or mild general reaction occurs. Upon repetition of the dose a more marked reaction occurs. The dose is decreased to 0.01 c. mm., and reaction follows again. At the next injection 0.005 c. mm. is given, again a reaction follows. Though the patient at first took 0.001 c. mm. without effect, now 0.0001 c. mm. may be followed by local swelling and soreness. This condition of increased sensitiveness is nearly always accompanied by symptoms of intoxication. As I have said, Löwenstein advises a rapid increase of dosage because he believes small doses, and particularly small doses long continued, favor the development of supersensitiveness. My experience does not confirm this view, for it indicates that supersensitiveness is commonly the result of overdosage and occurs particularly after severe general reactions. In pulmonary tuberculosis when increased activity of the disease supervenes an increase in tuberculin hypersensitiveness nearly always accompanies it.

When tuberculin treatment is carried on in the cautious manner previously outlined general reactions seldom occur, and severe general reactions are very exceptional. However, even with the greatest caution it is impossible to avoid general reactions completely. As long as they are mild no harm will be done. When general reactions occur tuberculin should be omitted for at least two weeks and then treatment be begun at a much smaller dose. Particular watchfulness is needed when approaching the dose that occasioned the reaction.

Should an intercurrent infection occur during treatment it is advisable to discontinue tuberculin temporarily until convalescence is established and then begin at a much smaller dose and again gradually increase the amount. During intercurrent infections tuberculin hypersensitiveness is variously influenced. During measles, as v. Pirquet has shown, hypersensitiveness is obliterated, to appear again during convalescence. Hamburger has noted a similar diminution of sensitiveness in pneumonia, diphtheria, scarlet fever, and cerebrospinal meningitis. However, during convalescence hypersensitiveness is often reestablished at a higher level than before the illness. Many authors have directed attention to the unusual frequency of conjunctival tuberculin reactions during convalescence from typhoid fever.

**The Terminal Dose**

The physician is now in full swing with tuberculin treatment. How long shall the treatment be continued and at what dose shall he halt?

From what has been said it must be evident that neither question can be answered directly. Tuberculin benefits accrue slowly and, since the
infection is chronic and at best heals but slowly, abrupt improvement cannot be expected. Nor, again, will a few doses of tuberculin accomplish appreciable results. Nor, yet again, as I have frequently emphasized, does any particular dose of tuberculin measure the benefit that has been obtained. I never advise tuberculin unless there is reasonable assurance that treatment will be persistently followed for at least six months. If conditions are favorable I like to give tuberculin continuously for from nine to twelve months. At the end of that period I prefer to stop treatment and to take it up again if it seems advisable after an interval of from three to six months. I can give no satisfactory reason for this preference other than clinical impressions, and I admit the ground for these is not very solid. Petruschky is a staunch advocate of intermittent treatment. He calls his plan the "etappen-kure." Treatment is administered for three months, then an interval of three months is interposed, again three months of treatment, and so on.

There is no absolute terminal dose, although custom has set certain precedents. Most observers cease raising the dose when 1,000 c. mm. is reached. Often this dose is exceeded. Denys has given as much as 10 c. c. B. F. However, the sum of clinical experience is that the average patient seems to lose ground when a dose of 1,000 c. mm. is exceeded. When this maximum is reached some clinicians advise repeating it indefinitely at ten- to fourteen-day intervals; others advise breaking off treatment at least temporarily.

Jochmann has sought to put the question of the terminal dose upon a more satisfactory basis. He proposes stopping the treatment at the point where the cutaneous tuberculin reaction is lost. He finds this point to be between 300 and 500 c. mm. O. T.

A course of tuberculin treatment extending over a period of from six to twelve months does not cure tuberculosis. Often the symptoms completely disappear, though the lesion persists. In other instances the lesion may be apparently healed, but we fear a fresh outbreak. Does a single course of treatment give all the advantages that tuberculin may confer? Again we must confess that we can give no more satisfactory answer to this question than to others that have been asked. However, most clinicians are in favor of repeated courses of treatment. I stand committed to this sentiment and feel that I have seen benefit follow the administration of tuberculin interruptedly over a number of years. Petruschky, Bandelier and Roepke, and Brown believe in applying the subcutaneous test some time after treatment has been stopped, and if the patient reacts advise another course.

If it is decided to give a second course of tuberculin, treatment may be pushed more vigorously. We find that as a general rule the tuberculin tolerance developed under tuberculin treatment persists for a very long time, often unabated for a year. Also we have gauged the patient's tol-
erance for tuberculin. Therefore, treatment may be begun at a higher
dose and the doses more rapidly raised. The following record illustrates
such a second course:

Elsie P., No. 7218, white girl, age 17 years. Came to the hospital April 26,
1911. Examination showed a fairly well nourished girl of healthy appearance,
with slight impairment and prolonged expiration over the right upper lobe and
a few fine râles at the base of the spine of the scapula. She had just returned
from the state sanatorium, where she had received tuberculin treatment over a
period of five months, having reached a dose of 0.03 c.mm. B. E. Tuberculin
treatment was resumed on April 28 with 0.0001 c.mm. B. F. On January 6 she
had reached 0.001 c.mm. and from this point on the dose was rapidly raised as
follows:

J une 9 B. F. 0.002 c.mm. No reaction.
June 13 " 0.006 " Slight local pain.
June 16 " 0.007 " No reaction.
June 20 " 0.009 " No reaction.
June 23 " 0.01 " No reaction.
June 27 " 0.02 " No reaction.
June 30 " 0.04 " No reaction.
July 7 " 0.07 " No reaction.
July 11 " 0.1 " No reaction.
July 14 " 0.3 " No reaction.
July 18 " 0.6 " No reaction.
July 21 " 1 " No reaction.
July 25 " 2 " No reaction.
July 28 " 3 " No reaction.
Aug. 1 " 5 " No reaction.
Aug. 4 " 8 " No reaction.
Aug. 8 " 10 " No reaction.
Aug. 11 " 15 " No reaction.
Aug. 15 " 20 " No reaction.
Aug. 22 " 25 " No reaction.
Aug. 29 " 30 " No reaction.
Sept. 5 " 35 " No reaction.
and so on.

REFERENCES

Comprehensive Presentations

1. Bandelier and Roepke. Lehrbuch der spezifischen Diagnostik und
   Therapie der Tuberkulose, 7th Ed., Würzburg, 1913.
2. Brown. Specific Treatment, Klebs, Tuberculosis, New York, 1909,
   508 to 565.
4. Hamman and Wolman. Tuberculin in Diagnosis and Treatment,
   New York, 1912.
5. Löwenstein. Tuberkulin zu therapeutischen Zwecken beim Men-
   schen, Handb. d. Tech. u. Meth. d. Immun., Kraus u. Levaditi,
   1908, l, 821 to 876.
REFERENCES


ORIGINAL COMMUNICATIONS

17. Behring. La thérapie immunisante à Marbourg contre la tuberculose, Tuberculosis, 1906, v, 343.
32. Denison. Two Years' Experience with Tuberculin British Congress for Tuberculosis, 1901, iii, 117.
REFERENCES

42. Freymuth. Über Anwendung von Tuberkulinpräparaten per Os, Münch. med. Woch., 1905, lll, 62.
43. Gabrilowitsch. Über des Endotin, die wirksame Substanz des Kochschen Alttuberkulins, Tuberculosis, 1901, viii, 507.
53. Hamman and Wolman (4) Section 1.
54. —— and ——. The Cutaneous and Conjunctival Tuberculin Tests in the Diagnosis of Pulmonary Tuberculosis, Arch. of Int. Med. 1909, iii, 307; ibid., 1910, vi, 690.
REFERENCES

76. ——. Immunization bei Tuberkulose, Deutsch. med. Woch., 1909, xvi, 1721.
79. —— Über die Agglutination der Tuberkelbazillen und über die Verwertung dieser Agglutination, Deutsch. med. Woch., 1901, xxxvii, 329.
80. ——. Über neue Tuberkulinpräparate, Deutsch. med. Woch., 1897, xxxiii, 209.
87. ——. Entfieberung mit Bazillenemulsionen, Zeitsch. f. Tuberk., 1910, xv, 284.
89. ——. Über innerliche Anwendung von Koch's Bazillenemulsion (Phytosoremid), Zeitsch. f. Tuberk., 1907, x, 508.
100. Liebermeister. Studien über Komplikationen der Lungentuberkulose und über die Verbreitung der Tuberkelbazillen in den Organen und im Blut der Phthisiker, Virchow, Arch., 1909, cvxvii, 332.
REFERENCES


122. ———. Die gegenwartige Stand der Tuberkulin-behandlung, Leipzig, 1901.

123. ———. Koch’s Tuberculin und seine Anwendung beim Menschen, Berl. Klin., 1899, heft 188.


131. Rabinowsitsch. Pathology of Tuberculin, Tuberculosis, 1913, vii, 479.


133. Roepke. Über diagnostische Tuberkulin dosen, Zeitsch. f. Tuberk., 1907, x, 412.
380 TUBERCULIN TREATMENT

REFERENCES


160. Vernet. Un cas de mennigite traite par la tuberculine Beraneck, Rev. méd. de la Suisse romande, 1907, vii, 562.


CHAPTER XIII

LEPROSY

RICHARD P. STRONG

MICRO-ORGANISMS CULTIVATED FROM CASES OF LEPROSY

Classification.—The subject of vaccine and serum treatment of leprosy at the present time is in a very indefinite condition, and judgment even of the approximate value of such treatment is somewhat difficult from a review of the reports of recent investigations carried on in relation to this question. This is particularly due to the fact that much confusion exists in regard to the etiological relation which the various micro-organisms that have been cultivated from leprosy bear to the disease. The bacillus lepræ was discovered in 1879 by Hansen in leprous lesions, and following his observations very numerous attempts have been made to cultivate this organism. In the past few years a large number of investigators have described the successful cultivation of various species of bacteria from leprosy, usually believing the one cultivated to be the cause of the disease. These organisms may be divided into 5 groups as follows, although some of them might perhaps be classified in more than one of these groups:

1. Partially acid-fast or acid-resistant diphtheroid organisms—the Babes-Kedrowsky type. At least 18 investigators have isolated micro-organisms which apparently may be included in this group.

2. Acid-fast organisms which produce yellow or orange-colored colonies. Five investigators have probably isolated organisms of this type, Clegg being the first to obtain a definite growth in pure culture.

3. Anaerobic acid-fast organisms isolated by Ducrey, Campana, and Serra.

4. Acid-fast bacilli which do not produce colored colonies. Five investigators, of whom Karlinski was the first, have claimed to obtain organisms of this type. Duval's recent work has been the most convincing regarding the etiological position of this organism.

5. Acid-fast streptothrixes isolated by Deycke-Pascha and Reschad-Bey, and Liston.

Wolbach and Honeij (1914), from a very complete review of the literature regarding the various organisms cultivated from leprosy cases, in considering the first four groups mentioned above, believe that there is no way of avoiding very serious attention to the significance of the
presence of the diphtheroid group, the pigmented acid-fast group, and the non-pigmented acid-fast group, in connection with the etiology of the disease. The number of times that each culture has been isolated, and the name of the investigator making the isolation may be summarized in the following table compiled largely from Wolbach’s and Honeij’s article with slight additions:

Diphtheroid organisms: Bordoni-Uffreduzzi, 1; Babes, 12; Spronck, 2; Gianturco, 1; E. Levi, 1; Czaplewski, 1; Teich, 4; F. Levy, 1; Barannikow, 2; Kedrowsky, 3; Klitin, 4; Bayon, 1; Williams, 5; Rost, 7;1 Shiga, 1; Duval, 1; Ophuls, 1; Wolbach, 1.

Acid-fast pigmented cultures: Rost, 7; Clegg, 16; Duval, 4.

Anaerobic bacilli: Ducrey, 1; Campana, 1; Serra, 3.

Non-pigmented, acid-fast cultures: Weil, 1; Karlinski, 1; Marchoux, 1; Twort, 1; Duval, 8.

Acid-fast streptothrices: Deycke, numerous cases; Liston, 1.

From the collected literature one may conclude that at least two, the diphtheroid and pigmented acid-fast, and perhaps all four varieties of the bacilli have been more or less commonly found in leprosy tissue. The diphtheroid organisms have been found in various parts of the world. In connection with the pigmented acid-fast bacilli, the careful experiments of Clegg and Duval are of particular importance. As Wolbach remarks, the possibility of the partially acid-fast diphtheroids becoming converted into completely acid-fast bacilli must be taken into consideration.

The employment of various serological tests for the determination as to which type of organism cultivated is the etiological factor in the disease has not led to any very definite results, though it is possible that progress along this line may be made in the future. Positive agglutination tests have been reported with human leprosy serum and the diphtheroid, anaerobic, non-acid-fast, pigmented acid-fast, and non-pigmented, acid-fast bacilli.

Kritchewsky and Bierger by means of the complement-fixation test have concluded that Kedrowsky’s bacillus is the true bacillus of leprosy, and that Duval’s chromogenic culture is not specific for leprosy. With Duval’s culture only 2 of the 28 lepra sera they examined gave a strong positive reaction, while with the Kedrowsky culture 24 of the sera gave strong complement-fixation which, however, was less marked in the cases with the nerve lesions of leprosy.

Kraus, Hofer, and Ishiwara have, by the employment of the bacteriolytic reaction, also attempted a differentiation of some of the bacilli cultivated from leprosy lesions. They found that the sera of different guinea pigs which had been inoculated with Duval’s and Kedrowsky’s organisms.

1 In Williams’ and Rost’s cultures it is stated that a non-acid-fast streptothrix gives rise to acid-fast rods, and a non-acid-fast diphtheroid which also produces acid-fast elements.
developed bacteriolytic properties which could be demonstrated by inoculating the specific serum and corresponding organism into the abdominal cavity of a guinea-pig. By this test these two cultures could be differentiated. Duval serum had no effect on Kedrowsky's bacillus, nor Kedrowsky serum on Duval's bacillus. Kraus, however, points out that it is not decided that either organism is the cause of the disease, and he failed to get a reaction with either in human cases of leprosy.

It is necessary to consider particularly in relation to the treatment of leprosy, the streptothrix, isolated by Deycke-Pascha and Reschad-Bey, first in 1905, and subsequently by Liston in 1912. Deycke-Pascha and Reschad-Bey by placing leprous material in saline solution and incubating for a long time succeeded in obtaining a growth of an acid-fast organism from a severe case of leprosy. At first the organism was not considered to be bacillus leprae. Later it was classified as a streptothrix. From a killed culture of this organism they prepared a vaccine and administered it to a patient from whom they had isolated the organism. A severe reaction followed the injection of the vaccine, and after repeated injections there was an improvement in the patient's condition. Believing that it was probable that the favorable effect noted in this patient was due to immunization with acid-fast constituents of the organism, they turned their attention to the isolation of this acid-fast substance. After many efforts they succeeded by fractional extraction with ether in securing a number of chemical products from the organism. Some of these they rejected as useless, and finally isolated a fatty substance to which the name "nastin" was applied. Nastin, as described, is a true neutral fat obtained from the "streptothrix leproides" which has been cultivated from different leprous nodules. More recently benzoyl chlorid was added for the purpose of dissolving the bacilli more completely. The new product thus formed was named nastin benzoyl, or nastin B, and it was stated that it did not cause the severe local reactions after injection as nastin alone had done. Nastin has been supplied as nastin B₀, B₁, and B₂. These products are supposed to be of different strength.

Uncertainty of the Successful Cultivation of Bacillus Leprae.—Notwithstanding the numerous recent observations carried on in relation to the cultivation of bacillus leprae, at the present time a number of investigators have not been convinced of the successful cultivation of this organism. Jadassohn, writing in Kolle and Wassermann's "Handbuch," 1913, upon this subject, is of the opinion that the organism has not been definitely cultivated in a practicable and usable manner.

Much work was carried on for several years by different assistants in the writer's laboratory in Manila regarding the cultivation of bacillus leprae, and it was pointed out some time ago by him that extreme care should be exercised in regard to the definite conclusion of the cultivation of this organism.
Fraser and Fletcher (1913) also incline to the belief that bacillus lepræ has not yet been cultivated. They made 373 inoculations of the bacilli, obtained from non-ulcerating nodules of 32 lepers, and the tubes were incubated for periods extending to more than 6 months, but no multiplication was observed except in a few instances where contamination occurred. Blood serum, placental and agar media, Duval's and Williams' modification of Rost's medium were among the media employed, both aërobically and anaërobically.

Diphtheroid bacilli were isolated, but were considered of no importance in reference to etiology on account of their ubiquity. Fraser thinks that the investigators who have described the transformation of a non-acid-fast into an acid-fast organism were deceived by transferring unwittingly lepra bacilli along with other saprophytes.

Bayon believes that Kedrowsky's culture is one of bacillus lepræ, and identical with the one obtained by himself, but that most of the other organisms which have been cultivated from leprosy lesions are not this organism.

Duval has suggested, upon the ground of serological experiments with immune sera from animals, that neither his non-chromogenic organism, nor the chromogenic one of Clegg is the same as any other known strain of acid-fast bacillus. In one of his most recent publications he believes that comparative biological studies indicate that the Clegg type of leprosy organism is closely related to the moist growing pigment-producing group of acid-fast saprophytes, while the Levi and Kedrowsky cultures, which are apparently the same, correspond in some respects to avian tubercle, and in others to Möller's smegma bacillus. The Rost and Williams culture he believes is identical with Grassburger's acid-fast saprophyte, while Karlinski's culture is not to be distinguished from Rabinowitsch's butter bacillus. He believes that bacillus lepræ has been cultivated by himself, and that there can be no doubt that the non-chromogenic acid-fast strain is the true leprosy bacillus, and states that the non-acid-fast streptothricial and filamentous forms which have been described as "stages" of bacillus lepræ by Kedrowsky and others have not been noted in any culture which he has isolated. He believes the organism of human leprosy is a bacillus and not a streptothrix. It must be admitted that at the present time there appears to be no unanimity of opinion as to which culture, if any, is one of the true etiological factors in leprosy.

Since there still exists so much confusion in regard to the etiological relationship of the various cultures isolated from leprosy cases, as might be expected, the favorable results obtained in treatment with various sera and vaccines obtained from these cultures are not very obvious.

1 In the discussion of this subject at the International Medical Congress, London, Aug. 23, 1913, further doubt is thrown upon the question by Duval, since it is stated that in his opinion there was still doubt if bacillus lepræ had been cultivated.
fore, in a consideration of the subject at the present time, it is perhaps more advisable to merely review the results which have been reported by the different investigators with the various sera and vaccines employed.

**SERUM TREATMENT**

In 1896 and 1897 Carrasquilla reported upon the successful treatment of lepers by means of a serum which he had prepared in the following manner: Blood was drawn from young lepers, allowed to coagulate, and the serum pipetted off. At intervals, from 50 to 100 c. c. of this serum were injected into horses, the animal being later bled, the serum collected and used for treatment. In the first report it was stated that 15 lepers had been cured by use of the serum. A number of investigators, Buzzi, Barillon, Alvarez, Arming, Atherstone and Black, Dehio, Grunfeld, Tidswell, Thompson, Medina, and Putnam, have employed Carrasquilla's serum in the treatment of leprosy. While temporary improvement has been noted in some instances, after its use the consensus of opinion at the present time is that this serum is of no value in the treatment of the disease. Babes in 1893 immunized animals with avian tubercle bacilli and injected the serum from such animals into lepers. In 1899 he prepared an extract from an organism isolated by him from leprosy cases, inoculated this organism into animals, and also employed their sera for treatment in human cases of the disease. No definitely favorable results have been obtained by this method of treatment. Abraham and Herman excised leprous nodules and subjected them to pressure, thereby expressing the fluid contents and lepra bacilli, diluted this fluid with normal saline solution, and injected it subcutaneously into horses, several cubic centimeters being inoculated every week or two for a period of 4½ months. Four weeks after the ninth injection the horse was bled, and the serum collected and used for the treatment of several cases of leprosy. With one exception no favorable results were noted.

Laverde obtained leprous nodules and used the tissue fluids from them to inoculate goats and donkeys. Patients were treated with the serum from these animals and the author states that marked improvement occurred in the leprous lesions, and a disappearance of anesthesia was noted. He continued the treatment for periods varying from 3 months to a year, and continued to produce improvement in 60 patients. Six of these cases, he stated, had been cured by this treatment. Further reports of its use have not been forthcoming.

Sugai, Mabuchi, Mononobe, and Ohashi obtained serum by inoculating goats with suspensions made from leprosy nodules. Only indefinite results were obtained by treatment of cases with this serum.

Metchnikoff showed that a serum produced by the methods of Carrasquilla and Laverde was cytotoxic rather than antitoxic or bactericidal in
its action, and that analogous effects are produced by the serum of a goat inoculated with normal human blood.

In 1912 Currie, Clegg, and Hollmann prepared a serum in horses by injecting at short intervals live cultures of acid-fast bacilli suspended in normal saline solution. The cultures had been isolated from lepers. Injections were given into the jugular vein in increasing doses, until finally 18 to 20 agar cultures were given at a dose. After the injections the animal became ill, and its temperature sometimes rose to 40° C. After several months' treatment of this kind the animal was bled, and it was found that its blood serum clumped the organism they had isolated from leprosy cases in dilution of 1-1,000, and strongly in a dilution of 1-500. No clumping occurred with bacillus margarin, bacillus smegme, or the grass bacillus of Moeller. The serum appeared to exert an inhibiting effect upon the growth of the organism with which it was prepared. The authors found that injections of this serum into patients suffering from leprosy did not, during the short period of time in which they used it, produce any beneficial results. They are not, however, without hope of increasing the potency of this serum to a point where it may be of benefit in the treatment of the disease.

Janin (1913) applied blistering fluid or plaster to portions of the skin of lepers in which the nodules were numerous, and injected 8 to 10 c. c. of the serum resulting, into the same or other patients. The first case treated was one of nodular leprosy of 5 years' standing. After 6 injections of the patient's own serum given at intervals of 10 days the lepromata disappeared and the skin regained its normal appearance. A second leper who had been suffering from the anesthetic form of the disease for 4 years was benefited by 3 injections of the serum of the first case. A subject of macular leprosy who was in feeble health improved considerably after 6 doses of his own serum obtained by blister. Another similar case received 4 injections after which the eruption grew paler and sensibility was restored in the more recent patches. Four injections of this man's serum were given to a girl who had been an anesthetic leper for 4 years. No change was noted in the lesions, but her health improved rapidly. Four doses of the same serum were administered to a man suffering from anesthetic leprosy of 10 years' duration and who had perforating ulcer of the foot. The ulcer healed and the patient became stronger, but the leprous areas of the skin remained unaltered. The author concludes that the blister exudate of lepers exerts a specific effect upon the course of the disease. A sharp febrile reaction sometimes occurs after the first injection.

Palldrock, in the treatment of 4 cases of leprosy, employed fresh complement-containing serum from animals together with salvarsan. He was led to make this experiment on the ground that the serum of lepers might be deficient in complement which has, however, been shown not to
be the case. Increasing doses from about 35 c. c. to over 100 c. c. were given subcutaneously, each patient receiving in all from 285 to 325 c. c. of serum. No benefit appears to have resulted from this method of treatment.

Dyer, influenced by the report of the condition resulting from the accidental biting of a leper by a viper in the West Indies, used the antivenom serum of Calmette in a series of leprosy patients with almost uniformly good results. Three of the patients recovered. Injections were made at frequent intervals, sometimes daily, and the dosage varied from 5 to 20 c. c. The buttocks and the shoulders were the usual sites of injection, though frequent injections were made in the lesions themselves, with the interesting result that these were directly influenced to favorable resolution.

Woodson has reported upon the same treatment with one case of leprosy which showed improvement, but the author doubted whether this was due to the serum alone.

**VACCINE TREATMENT**

Scholtz and Klingmuller recommended the tissue juice expressed from lepromata for the treatment of leprosy. Castellani and Woolley also employed a similar method of treatment. Woolley excised a nodule from the arm of a leper, ground it with sand in salt solution, centrifuged, heated to 65–70° C. for 15 minutes, and added enough carbolic acid to 5 per cent. The suspension was rich in bacilli. At intervals subcutaneous inoculations of .01 c. c. were made. Woolley later reported that no success had been obtained with this method.

Nicholls (1908) removed a leprous nodule together with a quantity of surrounding tissue. This was placed in a tube of glycerin bouillon and incubated for a fortnight. The broth and tissue were then slowly desiccated. The dried mass was finally powdered in an agate mortar, a suspension made, and the bacilli killed by heating to 60° C. and counted in a blood-counting apparatus. It was believed that during the time of incubation of the tissue the bacilli had multiplied therein. A case was treated with this substance which was said to contain 50,000,000 organisms per cubic centimeter. Under this method of treatment given every 4 days at first and later every 7 to 10 days, some nodules disappeared and others softened.

Rost in 1905 and 1909 prepared a substance known as leprolin from an organism which he stated had been cultivated from a case of leprosy. In a later report (1912) his method of preparation of the vaccine is as follows: Bacteria were removed from an agar slant culture of the organism, shaken up with distilled water, and centrifuged, the fluid being poured off and fresh distilled water added to the deposit, shaken up again,
and again centrifugaled several times so as to wash the culture and remove all external toxins. The deposit of bacteria, after final washing and centrifugaling, was dried and weighed and macerated with 7 per cent. glycerin and distilled water to make up a percentage solution. It was then placed in tubes and autoclaved. The tubes were then sealed and placed on a shaking machine for a period extending over several weeks. Ten minim of 1 in 400 of this vaccine produced a slight febrile reaction in cases of leprosy, and its therapeutic usefulness, according to the author, was very marked. Later another method of preparation of a vaccine was sometimes employed, the fatty substance of the bacteria being extracted by shaking in ether over a period of several weeks, filtering and centrifugaling the deposit, and evaporating the ether extract until it became of a sticky consistence and then adding olive oil to a weighed amount. Finally he prepared leprolin from 6 weeks' old bouillon cultures by filtering through paper and then sterilizing. Once to 3 c. c. are injected into the muscles every week. Of 30 lepers treated with his leprolin since 1909, 4 are said to have been cured and improvement has been noted in many others.

Whitmore and Clegg prepared a vaccine with the organism previously isolated by Clegg. The culture was killed by heating and suspended, and an attempt made to standardize it to 500,000 bacteria per cubic centimeter. The bacteria in this vaccine showed a great tendency to form clumps, on being allowed to stand without shaking. Injections were given once a week in doses varying from .25 to 1 c. c. of this substance. Any increase above this dose produced a local reaction preventing the absorption of the bacilli, and later an abscess would form at the site of the injection. Eleven cases of leprosy were treated in this manner for 8 months, and 21 cases for 7 months. None of these cases showed any improvement and the abscess production was considered a serious obstacle to the treatment. They next employed a glycerin extract from the organism isolated by Clegg, made in a similar manner to tuberculin. This substance gave no reaction in lepers analogous to von Pirquet's skin reaction in tuberculosis. Thirty-two cases of leprosy which had been previously treated with the first vaccine then received this substance. No reaction followed this treatment, and there was no improvement at the end of two months. They then made a preparation by emulsifying cultures of this same organism in 1-60 aqueous solution of sodium oleate, the bacteria being almost completely dissolved by this fluid. In 2 cases which were treated for 2½ months with this substance, no improvement resulted. The spleen of a leper which was rich in leprosy bacilli was ground up and the substance suspended in a 1-60 aqueous solution of sodium oleate filtered through cotton and heated for 1 hour at 60° C. None of the patients treated with this substance showed any improvement.

In 1912 Currie, Clegg, and Hollmann continued attempts of specific
therapy in leprosy, using in addition to serum the following preparations for treatment: (1) a vaccine prepared by practically the same method as previously described by Clegg and Whitmore; (2) by the injection of living cultures suspended in saline solution, inoculations of 1 c. c. being given at a dose; (3) inoculations of lepra toxin prepared from cultures of the leprosy bacillus after the method used by Koch in preparing the different tuberculins; (4) by extracting fatty substances from the cultures by chloroform and alcohol, prepared somewhat in a similar manner to nastin; (5) a few experiments were made with sensitized killed cultures, that is, cultures which had been exposed to the serum of monkeys previously injected with their leprosy cultures.

They conclude that: (1) Vaccine made in the ordinary way and administered subcutaneously cannot be employed advantageously except in very small doses, since any attempt to give large quantities results in abscess formation locally, and a very slow absorption. (2) While live cultures of bacillus lepræ have produced no beneficial results, they are deserving of further trial. They also produced abscesses unless given in small doses. (3) Toxins prepared from bacillus lepræ after the method of Koch's old tuberculin and his "B. E." appear to be of slight or no value in the treatment of leprosy. The extract consisting of the fatty material obtained from their leprosy cultures was not employed for a sufficient length of time to determine whether it was of value in the treatment of leprosy.

Williams, who regards his organism as identical with Rost's, as has been mentioned, cultivated a streptothrix from leprosy lesions and also prepared a vaccine, first by suspending the organism in olive oil or in salt solution after drying and powdering in a mortar. Later a 6 weeks' old bouillon culture of the organism (presumably in which the organisms were killed) was employed. This vaccine was used upon lepers accompanied by improvement in some of the cases.

Sandes, during 1912, treated 8 cases of leprosy by a suspension of killed "leprosy bacilli." The description of the culture is not given. At first 10,000,000 of the killed organisms were injected, and later the concentration of the bacilli was doubled, trebled, and quadrupled. No favorable results were obtained.

Turkhud, in 1913, prepared leprosy vaccine in the Bombay Bacteriological Laboratory from this same streptothrix isolated by Williams, and distributed to various physicians the vaccine for the treatment of leprosy cases. Fifty-nine cases of the disease were treated in various parts of the world, improvement being reported in 21 cases. The results vary with the observer.

Watkins-Pitchford noted no beneficial effect in 10 cases. Turkhud himself states that improvement in some cases in his experience is very definite, although marked and speedy improvement in every case has by no
means occurred. He states the injections must be repeated every 10 days for months. Sometimes a severe reaction results.

Rutherford treated 32 cases of leprosy occurring in natives of India with a vaccine prepared from Williams' culture. Ten of these patients disappeared during the period of treatment. Of the remaining ones the shortest period of treatment was 100 days, and 15 were treated for 153 days. Two cases remained unaltered in condition. In 3 cases it was impossible to decide whether there had been on the whole improvement or deterioration, and the remaining 15 cases grew worse. The author considers that the deterioration in these cases was probably usually due to the natural progress of the disease, and that the treatment did not effect it one way or the other. The vaccine was given usually in doses of 1 c.c. injected weekly.

Davies, 1913, has reported upon the treatment of a case of leprosy in a European girl, aged eight, with injections of an extract made from Bayon's bacillus. The maculae became red and inflamed a few hours after the injections, but soon improved. Six months later those on the body and limbs were almost invisible, but those on the face persisted, although they had faded to a great extent. The remedy was tried on 6 other lepers, but the results are not reported.

Bayon has treated 126 cases of leprosy by injections of a filtered diluted extract made from Kedrowsky's culture. He considers that the employment of a simple vaccine made of the bacilli killed, but not otherwise treated, can be of no service in this disease, since such organisms are not broken up in the tissues and no antibody formation can result. The extract from Kedrowsky's culture produces in early cases of the disease an intradermal reaction which may be used to confirm the diagnosis. The ultimate result of the treatment of the cases is not known.

Heiser has also reported in 1913 the cure of 2 lepers, both of whom had received vaccine treatment, but who appeared to be equally or more benefited by the other medical treatment which they had received.

**Treatment with Nastin.**—In regard to the treatment with nastin, many observers feel that the treatment is of no value, while others report in its favor. Among those observers who have obtained no favorable result may be mentioned Brinkerhoff and Wayson, Engel-Bey, Feindel, Jean-selme, Kinoshita, Kitasato, Lenz, MacLeod, Gordon, Messum, Montoya and Florez, Neish, Petra, Peiper, Rogers, Sadikoff, Sakaguchi, Ash-burton Thompson, Teague and Whitmore and Clegg. The results obtained by Anderson, Bichler, Neil Campbell, Chatterjee, Davidson, Gott-heitl, Jackson, Kiwull, Krikliwy, Kühne, Kupfer, Lie, Raschid, Rodriguez, Smith and Bisset, Williams, Wise, Ziemann, while not entirely conclusive, upon the whole seem to show that the remedy probably seems to influence the disease favorably. Only some of the more important results reported during the past year will be considered in this article.
Minett has treated 18 selected cases with nastin for nearly 2 years, and 6 for from 6 to 9 months, before further treatment with benzoyl chlorid was begun. These cases were compared with 71 unselected, treated only with benzoyl chlorid, and with 8 other cases left untreated. Each group included cases of nodular, anesthetic, and mixed leprosy. The author finds that with nastin alone very little beneficial effect was produced.

Schumacher has employed nastin in the treatment of 4 natives of German East Africa, all of whom suffered from mild skin lesions of leprosy of long standing. All 4 received subcutaneous injections, weekly at first, of nastin B₁ for 8 weeks, and then after 14 days' interval of nastin B₂ for 16 weeks. No general reaction was observed at any time and no reaction at the site of the injection. A favorable change occurred in the lesions of the skin and in the nasal lesions. Two months after the last injection the spots could be recognized only by small nodules which had become dark and softer. Lepra bacilli could no longer be found in the nasal discharge. Unfortunately the observation of the cases could not be continued longer.

Rudolph reports 6 cases of leprosy treated with nastin in which improvement occurred in all but one. In the last case treated the patient had been afflicted for 5 years, and incapacitated for 2 years, suffering from a mixed form of the infection complicated with iritis. In a course of 18 months he received 3 injections of nastin B₀, 8 of nastin B₁, and 12 of nastin B₂. After 6 months' treatment the iritis disappeared and the anesthesia was less. The lepromata on the hands and forearms became softer, but leprosy bacilli were still present. At the end of 18 months he had much improved. Bacillus lepræ was not discovered in the nasal secretion. The photographs taken before and after treatment afford convincing evidence of the improvement which took place.

Peiper records observations upon 31 lepers treated with nastin since the year 1907. Three are believed to have recovered, and six to have much improved under this treatment.

De Verteuil reports that in 2 anesthetic lepers an arrest of the disease occurred after 38 and 67 injections of nastin. In order to be successful the author states the treatment must be continued for 2 or more years. He believes nastin is contraindicated in ulcerating leprosy.

Wise and Minett, during a period of 4 years, treated by injections of nastin 244 unselected patients in British Guiana suffering from leprosy in various stages. Of this number at least 206 were under treatment for more than one year, and 118 for more than 2 years. Treatment was begun under the personal supervision of Deycke, who stayed some months in the colony, and afterwards the treatment was continued on the general lines laid down by him. The results obtained by the authors are not very encouraging. Some degree of improvement, they feel, is undoubted during the first 3 to 6 months, but this early improvement is a slight
one and only temporary. The condition retrogresses, the patient relapses, and the disease goes on as before. The experience in British Guiana shows that during the first 6 months of treatment there is a slight temporary check of the disease, but otherwise the natural course continues unchanged.

Scott analyzes the results in 49 cases treated by nastin, continued for considerable periods. Only nastin B, was used and a full tube was injected at each dose. The injections were given intramuscularly in the intrascapular region, the skin being sterilized with iodin. The results in the treatment are shown in the following table:

<table>
<thead>
<tr>
<th>Length of Time Under Treatment</th>
<th>&quot;Cured&quot;</th>
<th>Greatly Improved</th>
<th>Considerably Improved</th>
<th>Somewhat Improved</th>
<th>Stationary</th>
<th>Worse</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 years and over .............</td>
<td>1</td>
<td>..</td>
<td>..</td>
<td>..</td>
<td>..</td>
<td>..</td>
</tr>
<tr>
<td>2½ years and over ............</td>
<td>..</td>
<td>1</td>
<td>..</td>
<td>..</td>
<td>..</td>
<td>..</td>
</tr>
<tr>
<td>2 years and over .............</td>
<td>..</td>
<td>2</td>
<td>..</td>
<td>..</td>
<td>..</td>
<td>1</td>
</tr>
<tr>
<td>1½ years and over ............</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>..</td>
<td>..</td>
<td>..</td>
</tr>
<tr>
<td>1 year and over ...............</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>2¹</td>
</tr>
<tr>
<td>9 months and over .............</td>
<td>..</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>..</td>
<td>1</td>
</tr>
<tr>
<td>6 months and over .............</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>..</td>
<td>..</td>
</tr>
<tr>
<td>Under 6 months ...............</td>
<td>1</td>
<td>..</td>
<td>..</td>
<td>..</td>
<td>..</td>
<td>1</td>
</tr>
<tr>
<td>Total ..................... 49</td>
<td>8</td>
<td>12</td>
<td>12</td>
<td>10</td>
<td>2</td>
<td>5</td>
</tr>
</tbody>
</table>

"Cured" means complete restoration to health, strength, and working power, with loss of every symptom which causes inconvenience or incapacity. It does not mean in every case complete disappearance of every sign of leprosy.

In the opinion of the author the improvement noted in 85 per cent. of the cases constitutes sufficient ground for a very favorable conclusion. He remarks that the good effects are not rapidly striking. They are slowly and gradually developed, and are often not easily observed. They are nevertheless found to be substantial when treatment is sufficiently prolonged, and a careful estimate made of its results.

Non-specific Vaccine Treatment.—Many observers have also attempted to employ tuberculin in the treatment of leprosy. Since such a method of treatment is obviously not specific for leprosy it will not be considered in detail, but in general it may be stated that while improve-

¹ Intercurrent dysentery. Leprous symptoms had improved.
ment has occasionally occurred in some, no definite improvement has been obtained in the majority of cases. In a number of instances such treatment has been reported apparently to have been injurious to the patient.

A few investigators have also employed in the treatment of leprosy vaccines made from streptococci isolated from cases of erysipelas and one observer from yeast cells, but no beneficial effect was noted in the cases so treated.

**Spontaneous Cure and Improvement in Relation to Treatment.**—Numerous references are found in the literature to spontaneous cures among lepers and to cures by various forms of drug treatment. Some observers have believed that the disease is self-limited. It therefore should be borne in mind that in the treatment of leprosy by the various vaccines and sera considered above, errors in judgment are particularly liable to occur, and undoubtedly have occurred in many of the reports which have been referred to in this article. The irregular course which the disease pursues, sometimes with periods of temporary improvement and at others of retrogression, further increases the difficulty of determining even after an extended trial the value of a therapeutic agent.

---

**REFERENCES**

---. Ibid., 1913, xi, 201.
Carraquilla. Wien. med. Woch., 1897, Nos. 41 and 42.
---. Baumgarten’s Jahresber., xiii, 474.
---. Ibid., xiv, 438.
De Verteuil. Report by the Medical Superintendent of the Leper Asylum, Trinidad, 1913, iii, No. 4.
--- and ---. Ibid., 1907, No. 3.
--- and ---. Baumgarten’s Jahresber., xvi, 406.
--- and ---. Lepra Bibliotheca Intern., vi, 38.
Ducray. Giorn. Italiano della Mal. ven. e della pelle, 1892.
——. Lepra Bibliotheca Intern., vi, 49.
to March 31, 1913.
Karliniski. Verhandl. des viii Deut. Dermatol. Kongress, Sarajevo,
1913.
Liston. Scientific Memoirs by Officers of the Med. and San. Depts. of
the Gov’t. of India, 1912, No. 51, N. S.
Osler. Modern Medicine by Osler and McCrae, 1913, 532.
——. Lepra Bibliotheca Intern., v, 268.
——. Lepra, 1912, xii, 125.
Williams. Lepra, 1912, xii, 131.
CHAPTER XIV

SPECIFIC TREATMENT OF SYphilis OF THE CENTRAL NERVOUS SYSTEM

Homer F. Swift and Capt. Arthur W. M. Ellis, M.B.

GENERAL CONSIDERATIONS

Among the most important advances which have resulted from the study of syphilis in recent years is the remarkable increase in our knowledge of the syphilitic affections of the central nervous system. This additional information has affected particularly our conception of the adequate treatment of these conditions, since the realization of the frequency of the early involvement of the brain, cord, and meninges has established the necessity of considering this factor in the treatment of cases of early syphilis, while the discovery of the spirochæta pallida in the brain in general paresis has established parasyphilis as an active syphilitic disease, and has therefore altered completely our conceptions of the methods of treatment of these late conditions.

In the treatment of a general disease, even though the expression of its activity may be made evident only by signs of involvement of any special system of the body, our conception of the disease should not be confined alone to the disturbance of that system, but should be directed rather toward the disease as a whole. We should, therefore, consider syphilis of the central nervous system only as a part of the general disease syphilis, the various nervous manifestations of which are the results of the anatomical peculiarities of the brain, cord, and adnexa, and the various forms the disease assumes in its evolution.

Syphilis begins in a subject with no established immunity to the disease as a local infection which manifests itself as the chancre. The intense local tissue reaction of the primary lesion appears to depend on this absence of a general immunity, since, as a rule, none of the subsequent lesions show such an intense sclerosis. The absence of an early general immunity is also demonstrated by the possibility of reinfection, or, rather, superinfection, for a certain time during the early primary stage. Moreover, if such superinfection does occur a lesion results which is similar to
the first chancre. The effect of the general body resistance is manifested
also by the onset of spontaneous resolution in the initial sclerosis which
often occurs only after the outbreak of secondaries. The general distribu-
tion of the spirochete probably occurs soon after the individual has been
infected, and a "spirochetemia" may persist for several weeks or months.
Uhlenhuth and Mulzer (39) have studied the infectiousness of the blood
by injecting 2 c. c. of defibrinated blood into rabbits' testicles. If the
blood contains the infectious agent small nodules characteristic of syphilis-
tic orchitis develop in the rabbits' testicles in from forty to one hundred
and twenty days, and examination of the fluid obtained by the puncture of
the nodules shows the presence of spirochaeta pallida. By this method
they were able to demonstrate the virus in the blood in sixteen out of nineteen
cases of primary syphilis examined. Twenty-seven out of thirty-six
cases of early secondary syphilis also gave positive results. They demon-
strated also the infectiousness of the blood in some cases of latent syphilis,
the blood in two out of fifteen cases in this stage of the disease giving posi-
tive inoculations in rabbits. One of these latent cases was the mother of
a congenital syphilitic child born eighteen days before the blood was taken
for the test. She was probably infected at least three and one-half years
previously. Friihwald (13), in reviewing the literature upon the in-
fec tiousness of the blood in relapsing syphilis, reports eleven positive
results in the third to the twelfth month after infection, and two positive
findings in tertiary syphilis. Numerous trials with the blood of paretics
and tabetics all gave negative results until Levaditi (22) reported that by
means of the inoculation of rabbits he was able to demonstrate the circula-
tion of the spirochete in the blood of one patient with active paresis. The
results so far obtained show that the blood is most often infectious in the
early stage of the disease, and it seems probable that at this period most
of the dissemination of the virus occurs.

A certain period apparently elapses between the time of the general
invasion and the outbreak of general secondary signs, and, doubtless, dur-
ing this period is developed the first immunity or, rather, altered tissue
reaction, or allergy. The variations in the type of the cutaneous manifes-
tations illustrate well the change in the reaction between parasite and host.
The lesions are at first almost universal and very superficial, but with each
relapse they become more localized, tend to group, and involve deeper
structures, until finally the gummatous stage is reached. The change in
the type of lesion is probably due more to a changed tissue reaction than
to alteration in the spirochete, for superinoculation of syphilitic subjects
with a new virus, if successful, gives rise to local lesions which correspond
in general to the type of lesion usually present in the patient at that time.
The tendency of the spirochete is to be generally distributed throughout
the body, while the effort of the body is to localize its activity. The
expression of this effort is the altered tissue reaction or allergy. This
allergy is developed only after prolonged contact between host and virus, and although present to some extent in the early secondary period is most evident in the tertiary stage when the lesions are often limited to one or two areas.

These statements are strictly applicable only to untreated or poorly treated cases. Varying in proportion to the efficiency of treatment departures from this normal reaction of the body occur. It is a fairly old observation that relapses in patients who had received intensive mercurial treatment early in the disease were frequently of a more locally intense type than in untreated or poorly treated cases. This was especially true where treatment had been started early, before any general body immunity had developed. The treatment had interfered with that prolonged and extensive contact between virus and body cells which seems necessary for the development of a general defensive mechanism, and therefore, upon discontinuing treatment, the few remaining spirochetes which had resisted the mercury incited much more intense local reactions than usual. On account of this increased severity in the relapses many syphilographers in the mercury era recommended the postponement of treatment until after the outbreak of secondaries.

The course of syphilis has been thus briefly outlined that we may better discuss the manifestations of the disease in the central nervous system. While it has long been known that involvement of the central nervous system might occur early in the disease, it is only lately that the frequency and significance of this early involvement have been realized. For many years the dizziness, neuralgia, headache, heightened reflexes, and cranial nerve disturbances which have been noted in secondary syphilis were regarded as signs of meningitis. Ravaut (32) in 1903 showed that 67 per cent. of 113 cerebrospinal fluids from patients with early secondary syphilis exhibited some excess of protein or increased number of cells. Altmann and Dreyfus (1) examined the spinal fluid in 8 cases of primary and 56 cases of untreated secondary syphilis. Two of the fluids from the primary cases and 66 per cent. of the fluids from the secondary cases showed some increase in globulin or cells. We (9) have examined the fluids of 22 patients with early untreated secondary syphilis, and found 36 per cent. of them to contain an excess of globulin or cells, or both. Gennerich (14) has recently made a most extensive report based upon his studies of the cerebrospinal fluid in syphilis. Of 30 primary cases 8 had abnormal fluids, and one of the patients with an abnormal fluid had been infected only two weeks previously. One half of his early untreated secondary cases showed abnormal fluids, and the fluids from practically all of his patients in the early secondary stage examined before, during, and after intensive treatment, showed some increase in globulin or cells during the first course of treatment. He attributes this increase in pathological elements to a Herxheimer-like reaction.
SPECIFIC TREATMENT OF SYPHILIS

The Herxheimer reaction is best studied in the skin of a patient with fresh secondary syphilis. It consists of a focal erythema, involving the skin about the syphilitic lesions, which appears after an intravenous injection of salvarsan. This erythema is supposed to be due to an irritation produced by the "endotoxins" which are set free from the killed spirochetes. Gennerich has suggested that the increase in globulin and cells which he has noted in the fluid shortly after treatment is similarly due to "endotoxins" set free from spirochetes in the meninges. The fact that practically all patients with secondary syphilis showed this increase in cells and globulin led him to the conclusion that all of these patients had a syphilitic invasion of the central nervous system. In the light of our present knowledge of the infectiousness of the blood in the primary and early secondary periods it is hard to conceive how the brain, cord, and meninges could escape having spirochetes deposited in them.

The greater frequency of early cerebrospinal syphilis in patients who have received intensive but insufficient treatment is now easily explained. Practically all cases of early syphilis have an involvement of the brain or meninges. If they are untreated or receive only weak medication, such as mercury pills by mouth, the general body resistance is developed as a result of an extensive contact between body cells and spirochetes. This results in an involution of the meningeal lesions at the same time that the cutaneous lesions are disappearing. If, however, the development of an allergy is interfered with by more intensive treatment, and yet the treatment has not been sufficient to completely sterilize the body, those spirochetes which have survived the treatment will, when treatment is discontinued, cause severe local relapses in the tissues in which they are situated. It is well known that pathogenic micro-organisms which are situated in the meninges are less affected by curative agents than are micro-organisms acting elsewhere. This point will be discussed later. Because of this relative inaccessibility the spirochetes situated in the central nervous system not infrequently escape the sterilizing action of salvarsan, so that when the treatment is discontinued lesions appear in the meninges which at times approach the primary lesion in severity. At other times the process is more diffuse, as is shown by rather widespread signs. The cerebrospinal fluid of patients suffering from this so-called "neurorecidiv" shows a marked pleocytosis, marked excess in globulin, and a positive Wassermann reaction. Half of our patients with secondary syphilitic meningitis have had a negative Wassermann reaction in their blood serum, showing how well the syphilitic process is often localized in the central nervous system. Gennerich (15) has observed a few patients with meningeal "monorecidiiv" who were not treated immediately upon the appearance of symptoms. Although the Wassermann reaction in their blood was negative at the beginning, it reappeared in from four to six weeks, and secondary cutaneous manifestations similar to their first rash appeared at the time that
usually elapses after the first infection. In other words, the patients reacted as if they had been reinfected.

If this hypothesis of reaction between host, parasite, and treatment is correct, and the facts seem to be all in accordance with it, it would appear that insufficient intensive treatment in the early stages might be worse for the individual than none at all. It should not be inferred from this statement that intensive treatment should not be applied, but that our conception of what constitutes sufficient intensive treatment should be altered, and the condition of the cerebrospinal fluid be used as a guide to treatment as much or more than the Wassermann reaction in the blood. Only after the fluid has been normal for some time is one justified in discontinuing treatment. Abnormal fluids indicate the presence of a syphilitic inflammation somewhere in the cerebrospinal axis, and if treatment is discontinued too early these comparatively latent foci may suddenly become active and cause irreparable damage to important nervous centers or tracts. This important subject has been discussed in detail because it deals with the prophylaxis of later syphilitic disease of the central nervous system, which is even more important than treatment.

The subarachnoid space may be considered the great lymph space of the central nervous system. (Mott, 28.) From it extend the perivascular lymph sheaths which surround the vessels and extend along them as far as the capillaries. The lymph spaces surrounding the nerve cells (the so-called perineuronal spaces) are also in communication with the subarachnoid space, so that the cerebrospinal fluid, which Mott describes as the ambient fluid of the central nervous system, is in intimate relation with both the nerves and vessels of the brain and cord. This fluid, which may also be described as the lymph of the central nervous system, is not derived from the lymphatic vessels in the other parts of the body, but is secreted into the ventricles by the cells of the choroid plexus and passes outward from the ventricles into the subarachnoid space. The free communication between the subarachnoid space in the lumbar region and the ventricles is well illustrated by experiments of Dandy and Blackfan (7) with injections of phenolsulphonephthalein by lumbar and ventricular puncture. Phthalein injected into the ventricles appears rapidly in the lumbar fluid, and conversely phthalein injected into the subarachnoid space in the lumbar region travels to the ventricles and is present in the fluid obtained by subsequently tapping the lateral ventricles.

The early invasions of the blood and central nervous system with spirochetes has been mentioned. E. Hoffmann has designated the treponema pallidum as essentially a spirochete of lymphatic spaces, and the symptoms which result from its activity are due to the cellular reactions in the lymphatic vessel and tissue spaces. As above noted, the subarachnoid space may be considered as a great lymphatic space surrounding and in intimate relation with the central nervous system, and consequently it
is often extensively involved in the syphilitic process. The majority of cases of syphilis of the brain show extensive changes in the pia arachnoid in the region of the great subarachnoid cistern, which is situated at the base of the brain between the optic chiasm and the interpeduncular space. This region seems to be especially favorable for the pellulation of the spirochete and development of the syphilitic exudate. From this region the vessels of the brain take their origin, and many of the cranial nerves pass out. The syphilitic process often extends from this region along the perivascular sheaths of the vessels, and this perivascular inflammation extending toward the lumen involves the media and intima of the arteries. Microscopic examination of such vessels shows the most intense lymphoid cell infiltration in the perivascular space, with diminishing number of cells in the media, and still fewer in the intima. Similarly the spirochetes are found in great numbers in the outer portion of the exudate, and only a few can be seen in the intima. This perivascularitis is found in all stages of syphilis from early secondary syphilitic meningo-arteritis to general paresis, and is the most characteristic syphilitic lesion of the central nervous system.

In early secondary syphilitic disease of the central nervous system the process seems to be confined largely to the vessels and meninges, the region at the base of the brain being most frequently involved. This basal inflammatory process necessarily involves with great frequency the cranial nerves which pass out in this region, especially the auditory and optic nerves. Early there is usually very little actual involvement of the brain substance or of the cord, while, later, as the time of the tertiary period is approached, there is often more actual destruction of nervous tissue. This destruction of deeper structures is comparable to the gummatous stage elsewhere. Gumma developing in the meninges and pial sheath of the vessels extend into the brain or cord, producing granulomata of varying size, which cause symptoms according to their situation. In addition, small miliary gummatous may involve the walls of the vessel alone, or the periarteritis may extend inward so that there is, in addition, an endarteritis, with a narrowing or occlusion of the vessel and symptoms resulting from ischemic degeneration of the nervous tissue. There may also be a direct extension from the meningitis, so that meninges and nervous tissue are both fused in one exudate, but here the involvement of the nervous tissue is usually secondary to the meningitis, and probably the extension into the cord or brain is along the vessels, so we have what may be described as a meningo-arterio-myelitis. Lastly, a true endarteritis syphilitica, not secondary to a periarteritis, may occur.

Noguchi and Moore's (30) discovery of the treponema pallidum in the brain of paretics, which has since been confirmed by numerous observers, and the production of syphilitic orchitis in rabbits by injection of paretic brains, thus demonstrating that the spirochetes are present in an
active pathogenic state, has now established paresis as a form of active syphilis of the brain. The term parasyphilis has lost its significance, and only the convenience its use possesses in classification justifies the retention of the expression.

Paresis is a chronic diffuse meningo-encephalitis in which atrophy of the affected brain substance, leptomeningitis, and perivascularitis are the outstanding features, and accompanying endarteritis is comparatively rare. Not infrequently there is an endothelial proliferation and plugging of the capillaries supplying the cortex, thus compromising the nutrition of the parts involved. In addition, one often finds extensive plugging of the perineuronal spaces with small round cells; this also must certainly result in diminishing the nutrition of the cells. The distribution of the spirochetes in the brain in general paresis differs essentially from the distribution of the organisms in all other forms of cerebral syphilis, for, while in cerebral syphilis the organisms occur almost entirely in the meninges and in and about the vessels, in paresis they are only rarely found in these regions, but typically occur in the affected cortical tissue of the brain itself. This peculiar distribution has led Noguchi to designate paresis as a parenchymatous encephalitis in contradistinction to the interstitial type of inflammation which occurs in tertiary syphilis. McIntosh, Fildes, Head and Fearsides (37) have advanced the hypothesis that in so-called parasyphilis there is a hyperallergy of the parenchymatous tissue, which leads to a degeneration of this tissue when the spirochetes become active, while in tertiary syphilis the interstitial tissues are in a hyperallergic condition, and hence the inflammation is largely interstitial in type, with the degenerations as secondary manifestations. The observation that frequently in tabes and paresis the only previous syphilitic manifestation has been a slight primary lesion, and that there has often been an absence of secondary and tertiary manifestations, points to the possibility that the tissue reaction in patients with "parasyphilis" differs from that in patients who run the usual course with secondary and tertiary manifestations.

The early pathology of tabes dorsalis is not clear, because we rarely have the opportunity of examining the cord and brain until the disease has run its course, and nothing but degenerated nerve tracts remain. There is evidence that a meningitis involving the radicular nerves often exists, and the pleocytosis in the cerebrospinal fluid in active tabes points to the presence of meningitis. The conclusion that in the large majority of tabetics there is an active syphilis involving the cord, or meninges, or spinal ganglia, seems to be justified.

In the treatment of syphilis of the central nervous system, whether it be secondary or tertiary or "parasyphilis," whether the reaction is due to an interstitial or a parenchymatous hyperallergy, it is important to realize that the reaction is brought about by the activity of the syphilitic virus,
and is probably an effort on the part of the body to limit the activity of the spirochete. If, by means of drugs properly applied, we kill the spirochete and thus eliminate the stimulus to the inflammatory reaction we should arrest the progress of the disease, but only by complete removal of the spirochete can we hope to effect a permanent cure. The physical signs of nervous involvement are purely accidental, and depend upon the centers and nerve tracts implicated. If the degeneration is complete before treatment is instituted little, if any, change in the physical signs can be expected. If, on the other hand, the signs are due to pressure on nerve tracts, or interference with the nutrition of nerve centers, or if the symptoms are largely irritative in nature, much improvement may be expected from efficient therapy.

The Cerebrospinal Fluid in Syphilis.—The condition of the cerebrospinal fluid is one of the most important aids in diagnosis, and most certain guides in treatment of syphilis of the central nervous system. Because of the numerous ramifications of the subarachnoid space, and the intimate relation between the cerebrospinal fluid and the nervous tissue, perivascular lymph spaces, and meninges, it is probable that an inflammation involving any of these structures will be indicated at once by alterations in the fluid. These alterations involve: (1) number and character of cells; (2) globulin content; (3) presence of substance which binds complement in the Wassermann reaction; (4) type of curve given by the Lange gold reaction. In addition, it is well to note the pressure of the fluid, and at times the reduction of Fehling's solution. The information to be gathered from the examination of the last two factors is only slight, as the pressure varies considerably (in our experience between 90 and 200 mm. of water), and slight changes in the position of the body or emotions of the patient may have considerable influence upon it. High pressure indicates the presence of a fairly acute process or of considerable tumor (gumma) formation; very low pressures are not infrequently seen in paresis or taboparesis. Fehling's solution is reduced by most fluids; the only ones we have seen that did not reduce were obtained from patients with acute secondary syphilitic meningitis. The examination of the other four constituents is very important, and none of them can safely be neglected, as they are not absolutely dependent upon one another.

Pleocytosis.—The number of cells is probably a fairly accurate guide to the intensity of an existing meningitis. The highest counts occur in secondary syphilitic meningitis, where as many as 1,000 to 1,500 per c. mm. may be found. On the other hand, in endarteritis syphilitica the number of cells is usually normal, and in syphilitic spinal spastic paralysis, where the lateral tracts are supposed to degenerate secondary to syphilitic disease of the blood vessels supplying them, the counts are usually low, from 5 to 30. In tertiary cerebrospinal syphilis the count varies between normal and several hundred, according to the amount of meningitis. We
found an average of 86 cells per c. mm. in 21 cases in this stage; half of the counts were between 40 and 100. Likewise, in tabes the degree of pleocytosis depends upon the character of the individual case. If irritative symptoms are prominent the counts are relatively high; if the symptoms are of long standing and degeneration is predominant, or if there has been an arrest in the progress of the disease, the counts are usually low. In paresis the counts also vary considerably; while some cases show high counts of more than a hundred cells per c. mm., in the majority the degree of pleocytosis is moderate.

GLOBULIN.—The increase in globulin is probably an evidence of vascular disturbance in the central nervous system. It indicates an abnormal transudation from the vessels, and occurs in a great variety of conditions. Thus in all conditions causing pressure on the cord with resulting disturbances of circulation, such as tumor of the cord, meninges or bony canal one finds an increase in the globulin often of marked degree. Increased globulin is found also in the fluid of some patients with arteriosclerosis, and in all inflammatory conditions of the central nervous system and its supporting membranes. Like all inflammatory conditions, syphilis of the brain, cord, or meninges leads almost always to some increase in the protein content of the fluid, which, in some cases, is considerable. Every spinal fluid contains some globulin; whether in normal individuals this ever varies widely enough to approach the condition existing in disease is still unsettled, but if such exceptions do occur they are extremely rare, and it is probably essentially correct to regard a positive globulin reaction occurring in an individual in whom the conditions noted above, tumor, arteriosclerosis, etc., can be eliminated as certain evidence of an inflammatory process. The history of the individual and the Wassermann reaction in the fluid determine whether this inflammation is syphilitic or of other origin. The determination of globulin is especially important in the early stages of syphilis, since an increase in the amount of globulin in the spinal fluid may be the only evidence of invasion of the nervous system in a patient with primary or secondary syphilis. Usually, however, the increase in globulin is accompanied by some increase in the number of cells. The globulin is also increased, so as to give a definitely positive reaction by the Noguchi test in all of the other stages of syphilitic involvement of the central nervous system. In tabes of very long standing where the disease has been apparently quiescent for years it may, however, show no increase. The most marked reactions are found in some cases of cerebral syphilis and in general paresis, where the increase is usually extreme.

WASSERMANN REACTION.—While pleocytosis and excess of globulin are only signs of the existence of an inflammatory process in some part of the cerebrospinal axis, a positive Wassermann reaction in the fluid indicates the presence of active syphilis in some tissue bathed by the fluid.
It is established that there is no elimination of bacterial antibodies from the blood into the cerebrospinal fluid, and only a few chemical substances pass through the choroid plexus. The examination of fluids from patients with positively reacting sera and with active secondary or tertiary cutaneous syphilis, but without signs or symptoms of involvement of the central nervous system, and with cerebrospinal fluids otherwise normal, has failed to demonstrate a positive Wassermann reaction in the fluid. This shows that without involvement of the central nervous system there is no passage of the Wassermann "antibody" from the blood into the fluid. The presence of a positive reaction in the fluid, with a negative reaction in the serum, is not infrequent, so that the Wassermann reaction in the fluid is apparently due to a local production of reacting substances, induced by the action of the spirochete upon the tissues.

In testing the fluid for the presence of the Wassermann reaction the "auswertungsmethode" introduced by Hauptmann and Hoessli (17) should be used. In the interpretation of reports from the laboratory the physician submitting the fluid should know whether this method has been applied or not, for tests applied to large quantities of the fluid often yield information that is lacking when small amounts are used, while positive reactions with quite small amounts are also of prognostic importance, since they point to a more obstinate condition than positive reactions with larger quantities. The rate of diminution in the strength of the reaction gives a fair indication of the efficiency of treatment. The use of ten volumes of fluid, as compared with one volume of blood serum, does not affect the specificity of the reaction, in that it has not been found positive except where actual syphilitic infection of the central nervous system may logically be considered to have been present. For the past four years we have been testing the fluid in amounts corresponding to 2 c. c. and have occasionally detected positive reactions in this quantity when lower dilutions gave negative reactions.

In the early secondary period, when a slight increase in cells and globulin is found in so many of the cases the Wassermann reaction is usually negative. A positive reaction in this stage indicates a severe infection of the central nervous system. In this stage with positively reacting fluids there is usually a marked pleocytosis with signs and symptoms of a meningitis, localized or generalized. Positive reactions in this stage usually disappear after the application of vigorous anti-syphilitic treatment. In conditions, roughly classified as tertiary cerebrospinal syphilis, we have found that a positive reaction is given by about 95 per cent of the fluids if the larger amounts of fluid are used, while only about one-third react positively with the smaller quantities. Fildes and McIntosh (11), who used a very sensitive antigen and two volumes of fluid, obtained positive reactions in 90 per cent. of the fluids from patients in whom there was clinical evidence of involvement of both the brain and
cord, but only 26 per cent. positive reactions in patients with signs of
involvement of the brain alone. In both classes of patients 90 per cent.
of the serums reacted positively. In several of the patients with only
cerebral symptoms the pathological condition seems to have been largely
vascular, and it is a frequent observation that in syphilitic vascular dis-
ease, even involving the vessels of the brain or cord, the changes in the
fluid are often slight.

The fluid in tabes yields positive reactions to about the same extent as
in tertiary syphilis of the central nervous system. Only occasionally do
the tabetics with negatively reacting fluids show the disease in a more or
less quiescent state.

In none of the conditions classified under syphilis of the central nervous
system do we find the Wassermann reaction so uniformly positive as in gen-
eral paresis. Indeed, it is a marked exception to find a negative reaction
either in the blood or cerebrospinal fluid of a paretic, and if a patient sus-
ppected to be suffering from paresis has a negative Wassermann it is strong
presumptive evidence against the diagnosis. Furthermore, the fluids ob-
tained from paretics often react positively with very small amounts, so that
the determination of the point of disappearance of the reaction may help in
differentiating between paresis and cerebrospinal syphilis.

LANGE GOLD REACTION.—This test requires for its performance a
proper solution of colloidal gold, which is red in color. The spinal fluid
is diluted with 0.4 per cent. NaCl as follows: 1-10, 1-20, 1-40, 1-80,
1-160, 1-320, 1-640, 1-1280, 1-2560, 1-5120. Then the colloidal gold
solution is added, well shaken and allowed to stand over night, after
which the readings are made. With abnormal fluids the red color in cer-
tain of the tubes is changed. These changes are designated by numbers;
i.e., 1 red-blue, 2 lilac or purple, 3 blue, 4 pale blue, 5 colorless. Thus
the reading 5555421000 indicates that the first four dilutions were com-
pletely decolorized, the fifth dilution pale blue, the sixth lilac or purple,
the seventh red-blue and the next three unaffected. This is the typical
paretic curve in which the first four to six or more tubes show discolora-
tion. In the luetic type of curve there is not a complete decolorization
of the first dilution and the maximum change occurs in the second to the
fourth or fifth tubes, i.e., 3334310000, or 1221000000.

The luetic type of curve given by the fluid in cerebrospinal lues and
tubes is of confirmatory value. Its great usefulness, however, seems to
be in differentiating paralytic dementia from these two conditions. The
fact that the fluid from a patient with a clinical diagnosis of tabes or
cerebrospinal syphilis gives a paretic curve does not militate against it,
for it is well known that between ten and fifteen per cent. of tabetics
develop paresis, and at times it is with the greatest difficulty that the
differential diagnosis between cerebral syphilis and paresis can be made.
If future observations confirm what now seems most probable, viz., that
these paretic curve findings in the spinal fluids of patients, clinically not paresis, point to a possible development of the disease, it will be of importance in prognosis and of value in determining why symptoms of mental disorder appear in spite of vigorous and prolonged treatment.

**SALVARSAN IN TREATMENT OF SYPHILIS OF THE CENTRAL NERVOUS SYSTEM**

Salvarsan has now been in use long enough to demonstrate the comparative safety of its administration in any form of syphilitic involvement of the central nervous system, as well as in syphilis of the heart and blood vessels. Ehrlich's early statement that arsenic in the trivalent form is less organotropic than that in the pentavalent form seems to have been confirmed. In spite of thousands of patients treated, there have been practically no reports of optic atrophy, which is so frequent after the administration of atoxyl and arsacetin. Igersheimer (19) has shown that the toxic component of atoxyl and arsacetin is phenylarsenous acid, which produces a characteristic kidney lesion in dogs, a specific paraplegia in cats, and vestibular lesions in mice. These peculiar toxic effects have never been seen in these animals following the administration of salvarsan. By treating rabbits with syphilitic keratitis the same observer also showed that salvarsan has a true parasitotropic action. After such treatment both the diseased and normal eye were removed and tested for arsenic, and traces of the drug were found only in the diseased eye, which contained the spirochete.

In the past few years a number of patients with optic atrophy have been treated with repeated intravenous injections of salvarsan. In a number of them the atrophy has not progressed, in fact, the visual fields have increased. In others the retardation of a rapidly progressing atrophy has been reported. The chief danger from the administration of salvarsan to patients with syphilis of the brain is probably due to local Herxheimer reactions occurring in patients who have not been recently treated. Such reactions can be obviated by preliminary courses of mercury and iodids, and by instituting salvarsan therapy with relatively small doses.

Most patients with so-called parasyphilis seem to tolerate salvarsan treatment better than they do treatment with mercury. Sicard (35) lately reports that he has administered from 0.3 to 0.4 gm. of salvarsan, or an equivalent amount of neo-salvarsan, weekly to patients with tabes and paresis over a period of two years, so that 80 to 90 injections in all have been given. Numerous observers have reported the administration of 20 to 30 injections in one or two years.
Relative Value of Salvarsan and Neosalvarsan.—The inconvenience incident to the preparation of salvarsan was supposed to be obviated when neosalvarsan was introduced, but experience has shown that neosalvarsan has less therapeutic value. Castelli (17) showed that in experimental syphilis two units of arsenic in the form of neosalvarsan were required to do the work of one unit of arsenic in the form of salvarsan. Clinically, in the treatment of secondary syphilis, although the symptoms disappear equally well with either drug, the Wassermann reaction persists much longer in those patients who have been treated with neosalvarsan. In addition, more toxic manifestations have been noted after neosalvarsan in comparable dosage than after salvarsan, so one must conclude that salvarsan is the drug of choice. From the standpoint of expense and amount of arsenic introduced the balance is in favor of this preparation. The chief advantage of neosalvarsan is in the case of administration.

LOCAL TREATMENT IN CEREBROSPINAL SYPHILIS

While marked beneficial effects are frequently obtained from salvarsan combined with mercury and iodids, still in certain cases of resistant tertiary syphilis of the central nervous system, and in many of those patients suffering from what may be classified as parenchymatous syphilis, little or no effect on the Wassermann reaction in the fluid is noted at times as a result of this form of treatment. The desirability of some form of local therapy is obvious. In syphilis of the skin the local application of mercury often hastens the healing of the lesions. A similar local therapy would appear to be desirable in the central nervous system if it could be safely applied.

Attention has been drawn to the anatomy and pathology of syphilis of the central nervous system, and the fact that the choroid plexus holds back most of the drugs which are introduced into the blood stream. Most observers have failed to demonstrate any arsenic in the cerebrospinal fluid after salvarsan intravenously, so that in chronic syphilitic disease of the central nervous system most of the curative action of this drug is exerted on the side of the blood stream, and there is little, if any, application of therapeutic material by the cerebrospinal fluid, which is in much more intimate relation with the diseased tissue. Flexner (12) and his co-workers have shown the necessity of introducing therapeutic sera directly into the subarachnoid space in the various forms of purulent meningitis. The same principle no doubt holds good to a considerable degree in syphilis of the central nervous system.

Various attempts have been made to introduce mercury directly. Horsley suggested the washing out of the subdural space with 1-1000 bichlorid of mercury. Sicard (34) has used cyanid of mercury as follows:
Cyanid of mercury ...................... 0.1 to 0.2 mg.
Novocain ................................. 10 mg.
Salt solution ........................... 2 c. c.
Place in ampoule, seal, and sterilize by boiling.

He thinks that the effect of this is to increase the permeability of the meninges, rather than a local spirocheticidal action of the mercury contained in the preparation.

The question of local therapy by means of intraspinal injections of small quantities of salvarsan or neosalvarsan is at present in a very uncertain state. Experiments (10) with the use of such intraspinal injections in monkeys have demonstrated that in these animals the injection of even fractions of a milligram of neosalvarsan are highly irritating and, when used in doses of any size, may produce severe damage to the cord. Berger (3) has also shown in experiments on dogs the serious damage which may result from direct injections of neosalvarsan into the subarachnoid space. Twenty-five dogs were used in his experiments. He found that injections of 1 mg. of neosalvarsan in the cerebral subdural space were fatal for dogs in from one to five days. Five-tenths of a milligram produced a well-marked lesion at the site of injection, consisting of hemorrhage and cell infiltration, and even 0.2 mg. produced a similar lesion, though only of slight extent. Only with injections of 0.1 mg. or less was the site of injection free from histological lesions. He used the neosalvarsan in a dilution of 1-10,000. In the dogs in which the injections were fatal the pathological lesions consisted of hemorrhages into the subarachnoid space of the brain, miliary hemorrhages in the cerebral cortex and medulla, extensive hemorrhage in the medulla of the brain and cord, and areas of necrosis in the brain with cellular infiltration of the cortex and meninges.

Neosalvarsan has been used intraspinally also in the treatment of paretics. Opinions vary as to the advisability of the procedure. Marionesco (24) treated 13 patients, each receiving 5 mg. neosalvarsan in 4 c. c. of solution intraspinally. The injection in 8 was followed by permanent bladder disturbance. Marie and Levaditi (23) treated paretics with 5 to 40 mg. of neosalvarsan intrasinally. Some of their patients showed severe symptoms following the injection; others were entirely free from unpleasant symptoms. Ravout (33) claims that the reactions are due to the use of a hypotonic solution; he injects a concentrated solution prepared so that each drop contains 3 mg. of neosalvarsan. He has given 63 injections to 9 patients, and considers 6 mg. the best dose. Jeanselme, Verne, and Bloch (20) administered neosalvarsan intrasinally, using a solution with a concentration of 1 mg. per c. c. A dose of 0.25 mg. was first given, and the amount increased weekly until 5 mg. per dose was administered. They claim that these injections were not followed by any local meningeal reaction. In 3 of the 8 cases so treated there was con-
siderable diminution in pleocytosis, but no effect on the Wassermann reaction. Beriel (4), on the other hand, has seen severe symptoms—complete retention of urine, with subsequent incontinence, incontinence of feces, and finally a flaccid paralysis of the legs—develop following intraspinal injection of so minute a quantity as 0.02 cg. (0.2 mg.) of neosalvarsan. Another patient showed a severe reaction following a dose of 0.01 cg. (0.1 mg.). Marinesco (25) lately reports that he has administered from 3 to 12 mg. of neosalvarsan intraspinally without symptoms, provided the neosalvarsan is previously mixed with human serum. One patient out of twenty treated in this manner developed, however, a permanent retention of urine, followed later by infection of the bladder and death.

An analysis of the reports of patients treated intraspinally with neosalvarsan shows a great variation in intensity of resulting symptoms. While certain patients apparently tolerate comparatively large doses; others seem to be severely affected by very small amounts. Because of these unpleasant symptoms which follow intraspinal injections of neosalvarsan most clinicians in this country have given up this mode of treatment. Gennerich (15a) still injects fractions of a milligram in very dilute solutions. This author has become so enthusiastic over the results of intraspinal injections of these small amounts that he recommends that every patient in whom there is positive evidence of cerebrospinal syphilis should be treated in this manner. With such recommendations we disagree because (1) we feel that intraspinal treatment is not necessary in every case and (2) salvarsanized serum can be administered with greater safety.

The blood serum of patients treated intravenously with salvarsan has been shown to have definite therapeutic value when injected subcutaneously into patients with congenital and secondary syphilis. It (37) has also been shown that this serum exerts a spirocheticidal action on the spirochete of relapsing fever. When the serum is allowed to act upon the spirochete in vitro, and subsequently these are injected into mice, they do not develop in the mice as do untreated spirochetes or spirochetes on which normal serum has been allowed to act. Heating the serum to 50° C. for thirty minutes increases the spirocheticidal action. Gonder (16) has noted a similar spirocheticidal action of the blood serum of salvarsan-treated rats and hens. He has also found that the spirochætes gallinarum in infected ticks are killed when the ticks are allowed to suck the blood of salvarsan-treated hens.

These experiments demonstrate that the sera of salvarsan-treated patients have an antispirochetal effect. They can, moreover, be safely used for direct introduction into the subarachnoid space. The salvarsan content of these sera varies between 0.01 and 0.1 mg. per c. c.

Because of the uncertainty of the dose of salvarsan in autosalvarsanized serum, Ogilvie (31a) has recommended that a definite amount of
salvarsan be added to serum. In his and Fordyce's (12a) studies it has been found that the injection of this artificially salvarsanized serum is followed by distinctly beneficial results. It was further found that the dose of added salvarsan must be kept below 1 mg., or symptoms of myelitis of the lower part of the cord would appear. The dose now recommended is from 0.1 to 0.5 mg. The chief advantages of this method are known dosage and ease of preparation of serum for several patients at one time. One of us (Swift) has recently demonstrated that the most markedly spirocheticidal serum was furnished by adding a small amount of salvarsan to the serum of a patient who had received an intravenous injection of salvarsan. Another substance which has been recommended by Byrnes (4c) is the albuminate of mercury. When the dose is kept below 1 mg. repeated injections of this drug are reported to have been followed by beneficial results. In the preparation of this substance only human serum should be used, for repeated injections of horse serum may lead to the serious condition of hypersensitiveness or anaphylaxis to the foreign protein.

The observations (36, 38) on which the development of the use of autosalvarsanized serum was based are as follows: The cerebrospinal fluids of patients with tabes who had been treated with salvarsan intravenously, combined with mercury and iodids, showed a certain reduction in pleocytosis and in intensity of the Wassermann reaction. The addition of intraspinal injections of their own serum produced a rapid fall in the cell count and a disappearance of the positive Wassermann reaction. Control patients treated intraspinally with the serum of other patients showed a similar reduction to normal of the cerebrospinal fluid without the use of any other form of treatment. A series of patients treated from the beginning with combined intravenous and intraspinal injections improved fairly rapidly, so that after a year the fluid of a majority of them showed normal cell counts and a negative Wassermann reaction. The results of other observers (2, 6, 18, 26) have confirmed the above observations, and tabetic whose condition was stationary under salvarsan intravenously improved after the institution of intraspinal treatment.

The results of the treatment of paretics as far as permanent arrest or cure is concerned have been disappointing. There is no doubt that the number and length of remissions are much increased when a group of paretics is intensively treated. Many of these patients have been able to return to their work for prolonged periods after treatment had cleared up their abnormal mental symptoms. However, the disease shows a marked tendency to relapse and finally most of the paretics come to an unfortunate end. Some authors claim this is due to the failure of the serum injected intraspinally to reach the site of the active lesion in the cerebral cortex. For this reason intraventricular injections of dyes, such as trypan blue,
into the lateral ventricles is followed by a more intense staining of the cerebral cortex than when they are injected intraspinally. While theoretically the intraventricular injections of serum may be advised, it is difficult to repeat such treatment as frequently and as often as intraspinal injections can be given. Dr. Cotton in a personal communication has informed us that practically no better results are obtained from intraventricular injections than from intraspinal. The failure to cure permanently a case of paresis should not interfere with the undertaking of treatment, for the return of a paretic to his business or family, even if only for a year or two, may be of extreme importance.

The action of this serum when used for intraspinal treatment is not fully understood. At least four factors can readily be thought of which may play a rôle in its curative action. First, the salvarsan, even though in small quantities, when injected intraspinally may have a definite spirocheticidal action upon the organisms which are in the tissues. It is to be remembered that salvarsan injected intravenously may undergo changes in the body which render it more spirocheticidal, and there is much evidence which supports this idea. The use of normal serum to which equivalent quantities of salvarsan have been added immediately before intraspinal injection is not, therefore, strictly comparable. Second, there is a possibility that protective substances, which may be circulating in the blood serum, may be increased in amount and potency by the salvarsan injected intravenously. Intraspinal injection of these protective substances may be beneficial. Third, there is probably, after intraspinal injection of serum, an increased permeability of the tissues of the central nervous system. This increased permeability is doubtless set up by an aseptic irritation with consequent dilatation of the vessels and transudation of substances from the blood stream into the cerebrospinal fluid. Fourth, it is also possible that there is a beneficial effect produced by the inflammatory reaction which is set up by the serum. This beneficial effect may be compared to the Bier's hyperemia treatment of chronic infections elsewhere. The results of some work on the use of normal serum intraspinally, followed by diminution in the pleocytosis and, in some cases, in the strength of the Wassermann reaction in the fluid, strongly suggest that this non-specific reaction to an irritant plays an important part in the curative action of intraspinal injections of serum.

That intraspinal injections of serum are devoid of danger when proper aseptic precautions are followed seems to have been demonstrated, and more beneficial results seem to have been obtained by the use of combined intravenous and intraspinal treatment than from any method reported up to the present time.

The intraspinal injection of serum is not a substitute for other forms of treatment, but rather an adjuvant to them. In treating patients with syphilis of the central nervous system we should remember that we are
dealing with one of the most serious and stubborn forms of syphilis; hence, treatment must be both intensive and prolonged. Many patients show marked and sufficient improvement under salvarsan intravenously and injections or inunctions of mercury. Many patients with syphilis of the central nervous system have also syphilis of other organs, and in the tertiary stage iodids are of use in causing the syphilitic infiltration to dissolve.

_Technique_

A successful technique for the intraspinal injection of serum demands two main conditions: first, a knowledge of the principles and practice of bacteriological asepsis, and, second, the ability to do easily a successful lumbar puncture. The technique involved may be placed under four headings: (1) obtaining of blood; (2) preparation of serum; (3) lumbar puncture and injection of serum; (4) examination of spinal fluid.

_The Obtaining of Blood._—One-half to one hour after an intravenous injection of salvarsan the patient is bled and about 50 c. c. of blood obtained. This is most easily done by using a MacRae venous puncture needle,1 inserted in a rubber stopper, which is fitted into a 50 c. c. bottle-shaped centrifuge tube. The MacRae needle is a double needle provided with a shoulder which fits firmly against the upper surface of the rubber stopper. Immediately above this shoulder the shorter arm of the needle turns abruptly outward and ends in a round bulbous enlargement, over which a piece of rubber pressure tubing of fine bore can be passed. A sterile glass mouthpiece plugged with two cotton pledgets is inserted into the other end of the rubber tubing. By creating suction with the mouth through this tubing a partial vacuum is created in the tube and the flow of blood hastened. A supply of centrifuge tubes and glass mouthpieces, both plugged with cotton, can be sterilized by dry heat and then kept on hand for use as required. The MacRae needle, with its accompanying rubber stopper and the piece of rubber tubing, is sterilized by boiling immediately before use, and the rubber stopper carefully dried in a flame before inserting it into the centrifuge tube.

_Preparation of the Serum._—The blood obtained as described above is allowed to clot, and is then centrifugalized at high speed for about thirty minutes. The clear supernatant serum so obtained is then pipetted off into a large sterile test tube of about 50 c. c. capacity, and then diluted with the requisite amount of freshly prepared sterile 0.9 per cent. sodium chlorid. The usual dose is 30 c. c. to 40 c. c. of 40 per cent. or 50 per cent. serum; 40 c. c. of 50 per cent. serum, i.e., 20 c. c. of serum and 20 c. c. of 0.9 per cent. sodium chlorid is the dilution most often employed. It it is desired the serum may be injected without dilution with saline. In this case 15 to 20 c. c. of whole serum are injected. It is important

1 Tiemann & Co., 107 East 28th St., New York City.
that the serum after centrifugalization should be clear, and that, in removing it from the tube, the underlying sediment should not be disturbed. The presence of even minute amounts of blood cells or fibrin in the serum leads to unpleasant and sometimes alarming reactions, with pain, fever, and occasionally vomiting. Attention to this point is essential.

**Ogilvie Method.**—Serum is obtained from either an untreated or treated patient in the same manner as above described. Ten c. c. is pipetted into a sterile tube and to it the requisite amount of a weakly alkaline salvarsan solution is added and well mixed. This serum-salvarsan mixture is then incubated for one hour at 37° and afterwards heated to 56° C. for one-half hour, when it is ready for injection.

**Mercurialized Serum. Byrnes Method.**—To 12 c. c. of serum is added 1 c. c. of a solution of mercuric chloride in freshly distilled water, so made that each cubic centimeter contains 0.0013 gm. (1/50 grain) of mercuric chloride. To the serum thus prepared is added a sufficient quantity of normal salt solution to make a total volume of 30 c. c. If concentrated solution is used this step is omitted. It is then heated at 56° C. for half an hour, when it is ready for injection.

**Lumbar Puncture and the Injection of Serum.**—Lumbar puncture is performed with the patient lying on his right side in bed. The most essential procedure in the technique is the preliminary arrangement of the patient, who should be on the extreme edge of the bed, lying on his right side, and an attempt should be made to have the lower back as nearly straight in both planes as possible. The knees should be drawn up so that the thighs are at right angles to the trunk. Care should be taken to have the patient's shoulders straight, that is, both shoulders should be at right angles to the surface of the bed. This prevents a sagging of the body and consequent delignment of the spine in the lumbar region. In stout women a small pillow placed in the curve between the crest of the ilium and the costal margin is often of service. Attention to details in this stage of the procedure increases greatly the ease of lumbar puncture.

The intervertebral space at the level of the iliac crest is the one usually selected for puncture, but the space immediately above or below this may be used as desired. After sterilization of the surrounding parts the skin and underlying tissue in the area selected are anesthetized with a little 2 per cent. novocain. This makes a great difference in comfort to the patient, and is most essential where repeated punctures are to be carried out. The lumbar puncture needle is then inserted in the midline toward the lower border of the intervertebral space selected. The needle should be kept at right angles to the body in both planes when, if the patient has been properly placed, it should pass into the canal without touching bone in its course. If the space below the iliac crest is selected a slight cephalic direction of the needle must be used. The needle should be inserted until its point is felt to just touch the anterior wall of the vertebral canal. It is
to be remembered that nearly all unsuccessful attempts with such a technique are due to a misdirection of the point of the needle downward, that is, to the right of the canal. If, therefore, fluid is not obtained, the needle should be partially withdrawn and its butt strongly depressed; this raises the point of the needle, which then, on reinsertion, frequently reaches its desired destination.

The pressure of the spinal fluid is then estimated. A number of different forms of apparatus have been devised for this determination. The simplest consists of a graduated manometer tube of 3 mm. bore, to which is attached, by means of rubber pressure tubing of fine bore, a metal attachment, which fits accurately into the end of the lumbar puncture needle. When the needle is in place the trocar is withdrawn and the manometer attached, care being taken to lose as little of the fluid as possible. The pressure is then estimated by reading the height to which the fluid rises in the manometer. After the pressure has been noted the manometer is detached and the spinal fluid allowed to flow until its pressure falls to about 30 mm. This usually requires the withdrawal of about 30 c. c. to 40 c. c. of fluid. The serum is then injected by gravity. A simple apparatus for this injection consists of the barrel of a 20 c. c. Luer syringe (which has a capacity of about 30 c. c.), attached by means of a piece of rubber pressure tubing of fine bore to a metal attachment which fits accurately into the end of the lumbar puncture needle similar to that used in attaching the manometer. The rubber tubing should be about 40 cm. in length. The syringe, after attachment to the needle, should be depressed so as to allow the rubber tubing to fill with spinal fluid and so dispel all air. The mouth of the large test tube containing the serum should then be thoroughly flamed (in a gas or alcohol flame) and the serum poured into the barrel of the syringe, the top of which is then covered with a sterile sponge. On elevating the container the serum will flow rapidly into the subarachnoid space. All danger of increasing to a dangerous point the pressure in the spinal canal is avoided, since the greatest pressure which can be obtained by this technique is 450 mm. of fluid, a pressure which is far below the danger zone.

Examination of the Fluid.—The routine measures usually employed are: estimation of pressure, cell count, globulin reaction, Wassermann reaction, and gold reaction. The determination of pressure has already been described in the description of the technique of subarachnoid injections of serum. It remains to mention that the pressure varies widely, anything between 90 and 200 mm. being probably within normal limits.

Cell Count.—The preparations should be made as soon as the fluid is removed, as the cells settle rapidly and adhere to the sides and bottom of the tube if the fluid is left standing; they may be counted at leisure as long as sufficient time does not elapse for evaporation to occur. The counts are made with the pipette ordinarily used for leukocyte counts and
the Thoma-Zeiss counting chamber, Neuberg ruling. Two pipettes and two chambers are used in each estimation. For diluting fluid 10 per cent. acetic acid in distilled water is simplest. This is drawn to the “1” mark on the pipette, which is then filled to the “11” mark with spinal fluid. This gives a dilution of spinal fluid of nine in ten within the chamber. The fluid in the capillary portion of the tube is then blown out, and a drop of fluid from the chamber run on to the counting stage. The coverslip is then placed on the slide and, while firm pressure is being applied, pushed across the stage. With this method Newton rings are easily obtained on all four sides, thus assuring the constant depth of the preparation. The cells on the whole ruled surface are then counted. This ruled surface in the Thoma-Zeiss Neuberg-ruling chamber has an area of 9 sq. mm. As the chamber is only 0.1 mm. in depth, the total count gives the number of cells in 0.9 c. mm. of the diluted fluid. The figure so obtained must, therefore, be multiplied by 10/9 to give the number of cells per c. mm., and a further multiplication by 10/9 is required to correct the dilution; or, combining the two calculations, we can multiply by 100/81, that is, approximately 10/8. The result gives the number of cells in 1 c. mm. of undiluted fluid. The two chambers should correspond closely. Dreyfus (8) has suggested the following standard in considering the results of cell estimations:

<table>
<thead>
<tr>
<th>Cells per c. mm.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 to 5</td>
<td>normal</td>
</tr>
<tr>
<td>6 to 9</td>
<td>doubtful, border cases</td>
</tr>
<tr>
<td>10 to 20</td>
<td>slight lymphocytosis</td>
</tr>
<tr>
<td>21 to 50</td>
<td>moderate</td>
</tr>
<tr>
<td>Over 50</td>
<td>marked</td>
</tr>
</tbody>
</table>

**Globulin.**—Numerous methods are in use for the determination of abnormal amounts of globulin in the spinal fluid. A simple and satisfactory method is that of Noguchi (29). The reagents used in the Noguchi reaction are butyric acid (Merck) diluted to 10 per cent. in 0.85 per cent. sodium chlorid and a 4 per cent. solution of sodium hydrate. To 0.2 c. c. of spinal fluid 0.5 c. c. of butyric acid is added, and the mixture brought to the boiling point over a small flame; 0.1 c. c. of 4 per cent. sodium hydrate is then added, and the whole again heated to boiling. If carefully observed the reaction can be delicately shaded and relatively quantitative readings made. The tubes should be watched carefully for the first ten minutes, and read in half an hour, and again in two hours. If possible several fluids should be done at the one time for comparison. In reading the Noguchi reaction we adopt the following standard:

- ....................... opalescent to very faint haze
+ ....................... faint haze to haze
++ ........................ fine granular precipitate
+++ ........................ heavy granular or coarse flocculent precipitate
++++ ........................ very heavy flocculent precipitate
The Nonne-Apelt (31) ammonium sulphate method is even simpler than that of Noguchi, but is less delicate and, therefore, less satisfactory. This reaction is performed by mixing equal parts of a saturated solution of ammonium sulphate and cerebrospinal fluid; 1 c.c. of each is quite sufficient. It is advisable to layer the fluid on the sulphate solution; if the globulins are increased there occurs a more or less distinct ring at the plane of contact. After this preliminary observation the mixture is well shaken and the result may be read within three minutes. If it is distinctly opalescent or cloudy it is designated as a positive "Phase I" reaction.

The simplest method for the estimation of a protein increase is the Pandy test (31b), which is performed as follows: The reagent consists of a saturated aqueous solution of carbolic acid. To 1 c.c. of this reagent 1 drop of spinal fluid is added. The immediate formation of a bluish-white ring or cloud is the evidence of an abnormal protein content.

Wassermann Reaction.—The technique of this reaction has been discussed in part earlier in this article. Fuller details may be found in the numerous original papers which have appeared on this subject.

Lange Gold Reaction.—This test must be carried out in a well-equipped laboratory. From ½ to 1 c.c. of fluid should be collected in a separate clean tube. It is very essential that no blood or serum be in the fluid to be tested, and it is well to collect the last part of the fluid for the test so that the needle will be well washed.

The after-treatment of patients following spinal injections consists simply in rest in bed and amelioration of the pain of any accompanying reaction. The patient should be kept in bed for twenty-four hours after the injection, as in lumbar puncture, for any reason. The pains in the legs and back, which frequently follow intraspinal injections, are best controlled by the use of phenacetin and codein. Five-tenths of a gram of phenacetin and 32 mg. codein, repeated if necessary, is usually the maximum amount of sedative required. Morphia should never be required and never used in these patients.

Conclusions

Before undertaking the treatment of a patient with syphilis of the central nervous system the cerebrospinal fluid should be examined, and the number of cells, intensity of globulin, and strength of the Wassermann reaction noted. A preliminary course of mercury or mercury and iodids is probably advisable in many patients, as it will frequently prevent the appearance of local Herxheimer reactions. Salvarsan may then be given in courses of six to eight injections of 0.3 to 0.5 gm. each. At the end of such a course it is probably well to again examine the cerebrospinal fluid to determine the exact effect of treatment. If improvement has occurred,
especially in diminution in the globulin excess and strength of the Wassermann reaction, the mercury and salvarsan may be continued. If, however, no improvement has resulted, the institution of intraspinal treatment will probably be advisable. In patients with rapidly advancing tubes or with paresis the use of combined treatment from the beginning may be advantageously undertaken.

No rules can be laid down as to the length of period over which intravenous injections of salvarsan may be continuously administered. This varies with the susceptibility of the patient. It has been possible to treat some every other week for a year without unpleasant symptoms, but such intensive treatment is only called for in rapidly progressing cases which have not responded promptly to treatment. Usually it is advisable to give from four to six weeks’ rest after each course of six to eight injections, the individual injections being given every other week. Such courses may be continued indefinitely.

In treating the condition of tubes or paresis the slow disappearance of the abnormal constituents in the cerebrospinal fluid should not be cause for discouragement. If we remember that one does not hope to cure a patient with early secondary syphilis in less than nine months to a year of continued intensive treatment we should not expect to cure syphilis in a patient in whom the disease has been firmly established for from ten to fifteen years without a correspondingly greater effort. This effort is most certainly justified if we can obtain thereby a permanent arrest of the malady in a patient who has previously considered himself to be suffering from a progressive incurable disease.

REFERENCES

SPECIFIC TREATMENT OF SYPHILIS

27. McIntosh, Files, Head, and Fearnside. Brain, 1913, xxxvi, 1.
CHAPTER XV

PATHOGENIC FUNGI—SPOROTRICHOSIS,
OÝDIOMYCOSIS, INCLUDING BLASTOMYCOSIS, AND ACTINOMYCOSIS

T. R. BROWN

SPOROTRICHOSIS

The cause of this disease is now known to be due to a fungus to which Smith gave the name sporotrichum. The disease, when occurring in man, is known as sporotrichosis. Of this organism two species have been described: S. Schenki (Jordan and Perkins), and S. Beurmanni (Ramond and Matruchof). But since these two species vary only in minor details from one another they should be regarded as one and the same species.

This organism gives rise to a disease which is characterized by the presence of chronic subcutaneous abscesses which, in the course of five or six weeks, soften and break down. The disease is said to be characterized by the appearance of subcutaneous nodules with thickening of the surrounding lymph vessels. The nodules break down, forming multiple chronic abscesses, which, as a rule, finally heal. This pus is known to contain the infectious agent or virus.

The first case occurring in man was described by Schenk, 1898. Other cases were reported by Hektoen and Perkins, 1900. Since this time numerous other cases occurring in the human subject have been reported. A. Besson (1) says: "The parasite may infect the buccal, pharyngeal, and laryngeal mucous membranes. May produce 'gummatas' in the muscles and in the mammary glands, and also papular and vesicular dermatitis, osteitis, synovitis, and adenitis."

This disease, when occurring in horses, resembles somewhat epizootic lymphangitis, although it is readily differentiated from the true epizootic lymphangitis. The best discussion of the disease as it occurs in horses is given by Page, Frothingham, and Paige (5).

The etiological agent has been conceded by most observers to belong to a group of organisms known as molds or hyphomycetes. This group of organisms is, however, more or less indefinitely defined, and much confusion has arisen as to their proper classification. In botanical nomenclature the genera microsporon and trichophyton are synonyms of sporo-
trichum. On the other hand, bacteriologists and pathologists are inclined to use the term sporotrichum in a more restricted sense.

Organisms of the genus sporotrichum produce definite hyphae, which are more or less branched and possess a creeping tendency. Definite endospores have not been observed, or else they consist only of small side branches. The conidia are formed either on the sides or ends of the hypha, and may appear singly or in clusters. Conidia are usually numerous, ovoid or spherical in form, hyalin, and rarely colored. The hyphae are slender and septate and stain easily with the common anilin dyes. They are Gram-positive. Whether or not this organism ever develops a perfect or sexual stage is not known, but it does not seem probable.

Only one, or perhaps two, species of sporotrichum have been found to have any pathogenic significance, the more important one being

**Sporotrichum Beurmanni.**—Schenk in 1898 and Hektoen and Perkins in 1900 described a species of sporotrichum causing multiple abscesses in man. Since these observations many of the cases of this disease have been described in various parts of the world.

**Morphology and Methods of Detection.**—By direct examination of the pus the sporotrichum has the appearance of yeast cells representing spores or conidia, and measuring 3-6μ x 2-4μ. The study of colonies is best accomplished by the use of Beurmann and Gougerot’s method. The colonies are made up of mycelium and spores. The mycelia have a tendency to spread, forming long, delicate, colorless, septate, and branching filaments, measuring about 2μ in width. The spores or conidia are oval or fusiform in shape, and usually possess a brownish color. These spore bodies form at the end of the filament, and are usually from 5 to 30 in number. Some sugar media seem to inhibit the growth of mycelium, and only the yeast-like spores develop.

The parasite grows well on potato medium. Cultures when first isolated show growth at the end of about a week. Transplants at the end of two or three days show white filamentous colonies. These colonies enlarge, the center darkens, and finally turns dark brown or black, surrounded by a white margin. In old cultures the colonies finally become wrinkled. Liquefaction of gelatin usually takes place in about ten days. In gelatin stab cultures there is an arborescent outgrowth of filaments along the line of inoculation. The surface growth remains white. On agar and glycerin agar the growth is usually white. The filaments both on acid and plain agar or glycerin agar may penetrate below the surface. Blood serum is not liquefied. Growth occurs in litmus milk, but produces no change in the reaction of the medium. Coagulation of the milk occurs in some instances, but the medium does not become acid. Acid is produced in glucose, but not in lactose, saccharose, mannite, maltose, dulcite, adonite, inulin, or raffinose. This organism does not form gas in any of the carbohydrates. The organism does not grow well in sugar-free bouil-
Sporotrichosis

It grows somewhat better in plain and dextrose bouillon. A slight growth occurs in peptone solutions. The organism grows well in media six times as acid as the usual culture media. In such a medium any associated bacteria will die while the yeasts or moulds survive and multiply. In all fluid media the growth begins as a cluster of white filaments radiating from a point, which in time may settle to the bottom of the tube or become attached to the sides of the tube or to some particles and the remaining fluid may be left perfectly clear. These parasites do not produce indol or form nitrites. The organism is killed at a temperature of 60° C. for five minutes.

The microscopical appearance of the culture is best observed in a hanging drop of water or from a loopful of a bouillon culture. Staining shrinks the filaments, and stained preparations do not show the organisms as well as unstained preparations.

By means of the gelatin hanging drop one can observe the growth starting in different directions from one spore, and as the filament begins to fruit the spore remains in place instead of falling off. The filaments may show cross divisions, and in each filament there may be one or more round bodies.

Pathogenesis.—Rats and white mice are especially susceptible. Subcutaneous inoculations with pure cultures give rise to abscesses at the point of inoculation, which spread by extension. From these abscess formations the organisms can be recovered. Paraffin sections of abscesses show filaments of the organisms among the broken-down tissue cells. If intraperitoneal injections are made in male rats a double orchitis follows. There is an abundance of evidence to show that the human subject is susceptible to the disease, as is evidenced by a number of accidental laboratory infections that have occurred. The disease is usually transmitted by intimate contact with other cases, probably through abrasions in the skin.

Serum Diagnosis and Immunity.—It has been pointed out by Widal and Abrami that the serum from an individual suffering from sporotrichosis contains certain agglutinins which are capable of agglutinating the spores of the parasite. It is best to use a culture that has been growing for several weeks. The culture is ground up in a sterile mortar, and then an emulsion is made with normal saline solution, which is filtered through paper. The patient’s serum is mixed with this suspension and the determination made. Agglutination in dilution of from 300 to 800 usually takes place in about one hour. If the serum of a patient suffering from actinomycosis is mixed with this suspension agglutination may also be observed, but the titre strength of agglutinins is very much lower; rarely do we find it taking place in dilutions 1 to 80.

Complement-Fixation.—A number of investigators, notably Widal, Abrami, Joltrain, Weil, and Brussand, have shown that sporotrichosis also gives rise to certain specific immune bodies which can be demonstrated by
the ordinary methods of complement-fixation. Although many attempts have been made to produce vaccines, they have been of little or no value as curative agents.

Cutaneous Allergy.—Bloch has pointed out that a bouillon filtrate from an old culture, when applied to the skin of human subjects of the infection by the method of von Pirquet, will produce specific allergic reactions.

Oidiomycosis

Oidium Albicans.—A synonym of this organism is monilia candida. This parasite is the cause of thrush in infants. The same condition has been observed in young animals.

In 1840 Berg described this organism as the cause of thrush. Since that time numerous cases have been reported from which the organism has been isolated and cultivated. The disease is known in all civilized countries.

Morphology.—The mycelia are poorly developed; in some instances the whole growth consists of budding yeast-like cells, which may be spherical, elliptical, oval, or cylindrical, and may vary greatly in length.

In a number of instances there can be but little differentiation between conidia and the cells of the hyphae, but when artificially cultivated the conidia may be definitely adjoined from the top of the conidiophore. (Chlamydospores may form in the hypha.)

Isolation and Cultures.—The organisms may be easily isolated from the lesions of the disease. A slightly acid medium favors their growth. The organisms grow on moist media, forming superficial white, waxy, and granular colonies.

Pathogenesis.—Intravenous injections into rabbits produce a fatal infection, similar to infections associated with blastomycetes. By infecting the mouth of young animals and birds a typical thrush may be produced. The disease frequently occurs in the mouth of sucklings, forming white patches on the mucous membrane, which vary from mere points to areas of considerable size. The infection may involve the pharyngeal or laryngeal mucosa, but rarely gives rise to metastatic infection of the internal organs.

Blastomycosis.—A number of investigators, both in this country and in Europe, have described a chronic disease of the skin associated with yeast-like organisms. This disease may become generalized and produce fatal results. The post-mortem findings in generalized cases show that the internal organs and tissues are more or less involved. Both in the pus and diseased tissue round bodies with thick walls have been observed, which closely resemble yeast cells. Cultures made from the pus and dis-
Eased tissues show a blastomycete or yeast-like organism which is undoubtedly identical with the bodies observed in the pus and tissues.

MORPHOLOGY.—In the pus and diseased tissue these bodies are spherical in shape, measuring from 10μ to 15μ in diameter. These cell-like bodies are inclosed in a capsule-like membrane from .5μ to 1.5μ in thickness. These cells contain large and small granules. Budding forms have also been observed resembling budding yeast cells, and it seems quite likely that budding is the method of reproduction. Involution forms have been described having shrunken empty capsules of irregular contour. Mycelia do not develop in the lesions, but in artificial media the organisms undergo considerable variation with the production of a profuse mycelial growth. On artificial media at least three types of cell growth appear: (1) round budding cells, smaller than those appearing in the tissue; (2) segmented, branching mycelial threads, showing occasional budding; (3) aerial hyphae bearing conidia. These organisms grow readily on all cultural media. Perhaps the best description of the cultural characters of these organisms is given by Ricketts.

METHODS OF EXAMINATION AND ISOLATION.—In making an examination of suspected material it is best to take some pus from an abscess or scraping from inflamed indurated skin growth and macerate it in a 30 per cent. solution of sodium hydrate; this dissolves the pus and tissue cells, leaving the blastomycetes free. These blastomycete bodies are large, having refractile granular contents which are easily recognized. Since these organisms do not take the stain readily, it is better to examine them in unstained specimens.

To isolate these organisms in pure culture it is best to use saccharine or carbohydrate media. The organisms grow well at 37° C., but often quite slowly, and it is necessary to incubate the cultures for several days.

PATHOGENIC PROPERTIES.—Cultures recently isolated are usually much more virulent than older ones, although there seems to be considerable variation in the pathogenic properties of these cultures. Mice, guinea-pigs, and dogs are most susceptible of any of the lower animals to subcutaneous and intraperitoneal inoculations. The white rat, rabbit, sheep, and horse are somewhat less susceptible. The lesions of this disease may be localized in the form of abscesses, or, as in case of subcutaneous inoculations, a general infection may result.

Many investigators believe that blastomycetic organisms are intimately associated with carcinoma. Russell advanced the theory that malignant new growths are due to an infection with blastomycetic fungi. This theory, however, has largely been discarded. There are a number of facts which seem to disprove any relation of these organisms to carcinomatous conditions. When animals are inoculated with blastomycetic fungi the histological growths are not comparable to the conditions found in sarcomatous or carcinomatous tissues. Borrel states that “it is very difficult to explain the intracellular position of a yeast in an epithelial cell.” It has.
further been demonstrated that the serum of an individual suffering from a malignant growth does not contain any agglutinins for yeasts.

TREATMENT.—At present very little has been accomplished in developing a specific therapy in this disease. Iodid of potassium or copper sulphate has been used with some success. Salvarsan has been used with reported favorable results (Simpson).

Specific immunity to this infection is still imperfectly understood. Ricketts and Eggers prepared a number of vaccines by grinding the organisms and making suspensions. With these suspensions they immunized animals, and were able to produce agglutinating and precipitating antisera. The practical value of these vaccines in cases of the disease has as yet not been definitely determined. Christensen and Hektoen have used these vaccines in two cases, but since these cases were so far advanced no definite deduction could be made.

Theoretically it would seem that this vaccine treatment should be of value, but much work still remains to be done on the question of vaccines in connection with this disease.

ACTINOMYCOSIS

Actinomycosis is a disease which affects man in common with certain of the domestic animals, occurring, however, more frequently in oxen, swine, and horses. This disease is of special interest in that it is a microbic affection in which the parasite belongs to the higher order of bacteria. The term streptothrix is sometimes used as synonymous with the terms nocardia and actinomyces, but this usage is not correct.

Actinomyces includes those types which show true branching, and in which spore formation has not been observed. It is quite likely that nocardia and actinomyces represent a single genus. The parasite of this disease was first isolated by Bollinger, and described by him in 1877. Harz, the botanist, first applied to this disease the name actinomyces, or ray fungus, because of its peculiar rosette-like appearance. In 1878 Israel (3) described the parasite in the human subject, and a year later Ponfick identified this organism as the same as that found in the ox. Since the first case was noted in man a large number of cases have been observed in the human subject, and we now know that the disease occurs more frequently in man than was formerly supposed. Whether or not there exist various strains of actinomyces seems to be an open question. Observations of Wolf and Israel, together with those of Wright, who isolated thirteen different strains, seem to indicate that most, if not all, the cases clinically observed are due to one and the same organism.

A number of distinct species of streptothrix have been isolated from
cases of the disease in man where the lesions resembled more or less closely those of actinomycosis.

The term actinomycosis should be restricted to a group of organisms having morphological characteristics in common with bacteria, on the one hand, and the hypomycetes on the other. They resemble bacteria in that they develop homogeneous threads in cultures. These threads become segmented, and may appear as short bacilli or coccus-like fragments. They differ from moulds in that they do not have a double wall, and are not filled with fluid-containing granules. The segments are separated from one another by a distinct partition. They resemble moulds in that they develop from spores into dichotomously branching threads. These threads ultimately form colonies and resemble more or less true mycelia. Many of the threads composing such a mycelium become fruit hyphae, which later break up into round, glistening, spore-like bodies. As a rule these spores do not have as much resistance to heat as bacterial spores. They are readily stained by ordinary methods. This whole group of organisms has not been definitely defined, and many of the morphological characters have not been sufficiently worked out to justify a logical classification.

The groups of actinomyces which have been most carefully studied are A. bovis, A. madine, A. farcinicus, and A. epipteri; although numerous other varieties have been observed in association with important and interesting pathological lesions. Many of the pathological lesions in which actinomyces have been observed show in many instances certain similarities to true tubercular processes, and in some cases, save for the absence of tubercle bacilli, are indistinguishable from tuberculosis. Many varieties of actinomyces have been encountered in abscess of the brain, cerebrospinal meningitis, endocarditis, bronchopneumonia, pleuropneumonia, pustular exanthemata, abscess of the lung, bronchiectasis, pulmonary gangrene, necrosis of the vertebrae, subphrenic abscess, noma and pseudo-tuberculosis. Unfortunately these organisms have not been thoroughly studied, and little is known of their morphological and cultural characters. This is due, perhaps, to the fact that it is somewhat difficult to isolate these organisms from diseased tissue, and likewise because cultures once isolated do not always produce the characteristic pathological conditions when injected into susceptible animals. In a microscopical examination of the tissue these actinomyces may appear as long, convoluted, irregular staining, beaded, branching threads, or in clumps of short characteristic beaded and sometimes branching rods. Long or short threads have been encountered which give a distinct impression of mycelial structure. Varieties have been described in exudates which take the Gram stain, while others are decolorized by the process. Some of them are to a limited extent resistant to the decolorizing action of acid, but may be rendered visible by the ordinary method of staining. Many of these irregular types have been described, but their morphological
characters have not been sufficiently studied to enable one to definitely identify them.

**Microscopical Appearance of the Parasite.**—In tissue actinomycetes appear in small round masses or colonies, which, when fully developed, are easily observed with the naked eye. The largest of these bodies are about the size of a small pin head. In cases where there is a considerable amount of suppurative these bodies are found lying free in the pus and are easily recognized. If a small quantity of this pus is placed on a slide and held up to the light these particles are plainly visible. They may have a distinctly yellow color or greenish-gray tints, or they may be transparent or jelly-like. They may also be opaque and give rise to various shades of color, such as white, yellow, greenish, or almost black. They are usually soft, and sometimes have a gelatinous consistence, although at times, and especially in cattle, they are hard and gritty, owing to the presence of calcareous deposits.

**Gross Appearance.**—These parasites, which may be regarded as belonging to the streptothrix group of higher bacteria, present pleomorphic characters. The colonies which grow in the tissue give rise to distinct morphological characters, such as filaments, coccus-like bodies, and clubs.

These filaments are usually thin, and are often of great length, measuring about 0.5 μ in diameter. They are composed of a central protoplasm inclosed by a sheath. The older filaments may contain granular protoplasm, and they occasionally contain dark pigmented granules. They also show branching characteristics which readily distinguish them from the ordinary bacteria. In the early stage of development the colonies are made up of filaments loosely arranged, but later these filaments become so compact that their structure cannot be made out. In the younger colonies these filaments usually take a uniform stain, while the older colonies take the stain more or less unevenly, and give the appearance of chains of bacilli or cocci. The sheath enveloping them is usually easily distinguished.

**Coccus-like Bodies.**—Actinomycetes, like other species of the streptothrix when grown on culture medium, show on its surface filaments growing upward, which segment into rounded spores of gonidia. Under natural environment outside of the body these gonidia become free and form new centers, giving rise to filaments. They have a much greater power of resistance to heat or disinfectants than the filaments, but less than the spores of most of the lower bacteria. These filaments and spheri-
cal bodies are readily stained by Gram's method.

**Clubs.**—At the periphery of these colonies are formed club-shaped or pear-shaped bodies, which appear to be a sort of hyalin enlargement of the sheath at the outer end of the filament. These bodies possess a homogeneous, uniform structure. These club-like bodies are very delicate when obtained from the human subject, and easily break up, and are often soluble in water. These bodies are frequently recognized in the examination
of the fresh materials, but are not to be recognized in the hardened specimens. They do not take the Gram stain, although in very young cultures some bodies which are faintly Gram-positive may be observed.

Lesions.—In man the parasite produces a chronic inflammatory change, and usually ends in a suppurative which slowly spreads. In some instances there is a considerable amount of granulation tissue formed, and the mass feels solid, although there may be a little softening in the center. This condition may exist in the subcutaneous and dense fibrous tissue, especially so if the disease is not far advanced. In cases where the internal organs are involved suppuration is the most prominent feature. In the liver we find multiple foci of suppuration, and these areas present a honeycomb appearance which is quite characteristic of the disease. The colonies of the parasite may be seen with the naked eye. After the disease has been in progress for some time these colonies form large areas of suppuration. The pus is usually greenish-yellow in color and somewhat slimy in character.

In cattle and the lower animals the condition is somewhat different. There is usually an abundance of granulation tissue, which may result in large tumor-like masses, which are more or less undulating in character, and may contain well-developed fibrous tissue containing areas of new formation in which irregular abscess formation is usually present. The cells immediately surrounding the colonies are usually round, although they are frequently columnar in shape and concentrically arranged. Leukocytes or granulation tissue may invade these actinomycete colonies, and portions of the parasites may be found within the leukocytes or within small giant cells which are present. It is not common to find in human actinomycosis that the leukocytes have invaded the older colonies.

Distribution of Lesions.—In man the lesions may occur in almost any part of the body, due to the many avenues of infection. In many instances the parasite gains entrance by way of the mouth, either through some abrasion or by crypts of the tonsils, or it may find a favorable environment around a decayed tooth. At the portal of entry there are often swelling and suppuration, from which the disease may be spread in various directions. The periosteum of the jaw or vertebrae may be involved, resulting in caries or necrosis. The pus may spread to the deeper tissues of the neck. In a few instances the primary lesions occur in the intestine, usually the larger intestine. In such cases the parasite more than likely enters the tissues from the esophagus. The parasite may penetrate the wall of the intestine and set up ulcerations, followed by necrosis. The infection may spread to the peritoneum or to the extraperitoneal tissue. The retrocecal connective tissue may be involved, and is frequently the seat of suppuration. An interesting condition may occasionally occur in the intestine, where slightly raised plaques have been found. In the large and small intestines these are composed of epithelial
PATHOGENIC FUNGI

cells and actinomycetes. The primary lesion may also be pulmonary or peribronchial where there follows extensive suppuration of the lungs, when the parasite enters by way of the respiratory passages.

Infections also occur where there have been abrasions of the skin. Grainger Stuart and Muir have reported cases where the genital tract has been involved, in which both ovaries and both Fallopian tubes were affected. After the parasite has once gained entrance to the tissues by means of any of these channels the internal organs may be infected. The organ usually attacked is the liver, although abscesses may occur in the lungs, kidneys, brain, etc. In such cases the disease is spread to the various parts of the body by the blood stream.

In case of the lower animals, and especially in cattle, the disease is more or less localized. Tumor-like masses appear in the region of the jaw or neck. The palate or tongue may be affected. In the latter there are usually considerable enlargement and induration, followed by nodular thickening on the surface, and this condition is known as "woody tongue."

Occurrence of the Parasite in Nature.—Since this disease is most common among the lower animals, it is interesting to inquire where these parasites are found in nature. We know that the mouth or the digestive tract is usually infected, and it would seem that the parasite is associated with the food. A considerable amount of evidence seems to indicate that actinomycetes grow on grains, especially on barley. In both cattle and swine the parasite has been found growing around fragments of grain embedded in the tissue. In the human subject a number of cases are reported in which there is history of penetration of the mucous membrane by particles of grain or straw. There have also been a number of human cases which seem to have contracted the disease directly from infected animals. The observation has been made that animals pastured upon low lands and in river valleys are more liable to contract the disease than those feeding upon high and dry ground. It has also been observed that cattle fed upon rough or coarse fodder are more prone to the disease on account of abrasions of the buccal mucosa, than those kept upon less harsh food.

Actinomycetes grow slowly when cultivated on artificial media, and while growth takes place best at 37° C. they will develop at room temperature. On agar or glycerin agar at 37° C. growth is usually visible on the third or fourth day. At first only drop-like colonies appear. They gradually enlarge and take on a reddish-yellow tinge. These colonies adhere closely to the surface of the medium and are difficult to break up. The older growths take on a dusty brownish-yellow appearance. The organism grows well under anaerobic conditions on agar. The growth on potato is quite similar to that on agar. On gelatin stab the surface growth appears as a raised round colony. The gelatin is slowly liquefied. In very young cultures the growth consists of branching filaments, which are easily and uniformly stained; older cultures, and especially the superficial filaments, show segmentation into gonidia. True clubs are not formed in artificial cultures, although it is not infrequent to find a considerable enlargement at the end of the filaments.
Pathology.—Actinomycosis is a disease which occurs spontaneously, and its connection with other cases is not always easy to explain. It occurs most frequently among cattle and human beings; horses, sheep, dogs, and cats may also be affected. It seems to have a predilection for the parts adjacent to the mouth and pharynx. It is not infrequent, however, to find it in the lungs, intestinal canal, and upon the skin. The lower jaw is most frequently affected, where the disease is first noted by a hard nodular swelling, which is later followed by softening and necrosis. The bone is frequently involved, causing a rarefying osteitis. These swellings break down and sinuses are formed from which pus is discharged. There is a formation of epithelioid cells and small round-cell infiltrations about the filamentous knobs or granules. In old cases the affected part may be surrounded by connective tissue, and the necrotic masses may become completely calcified and a cure result. Infections of the lungs or of the intra-abdominal organs are much more serious. When early death occurs it is in most cases due to secondary infection.

Diagnosis.—An accurate diagnosis of actinomycosis cannot be made unless the parasites can be detected in the pus. In all cases when this disease is suspected a careful and painstaking search should be made to determine the presence of this organism. These colonies can be recognized by the naked eye if some of the pus is spread out on a glass slide. If one of these colonies is washed in salt solution and examined unstained, the clubs, when present, are readily seen on a microscopic examination. These colonies may be stained with any of the anilin dyes, but perhaps the best pictures are obtained with microcarmin. In order to study the filaments it is essential that the colonies be broken up, then stained with any of the basic anilin dyes; a dilute solution of carbol-thionin-blue yields the best results, but in the case of sections it is best to use Gram’s solution to bring out the filaments, and then counter-stain with rubin in order to distinguish the clubs. By this method very good specimens can be obtained. In order to isolate the parasite the medium should have a reaction of about 1.5 per cent. acid to phenolphthalein. One per cent. glucose broth or glycerin agar seems to give best results. Cultures should be made and incubated anaerobically and aerobically at 37° C. Unless the pus is free from contamination with other organisms it is difficult to obtain pure cultures because of the slow growth of the actinomyces.

Treatment.—Very little is known concerning immunity. In spite of the fact that much work has been done in the way of vaccine therapy, nothing definite has been accomplished. In a number of cases of actinomycosis iodid of potassium has been used with marked success.

[Wynn (8) has reported a case of empyema due to actinomycotic infection in a boy of 14 years. Following drainage, the patient received inoculations of a preparation of cultures of actinomycetes (actinomycotin). The improvement noted was ascribed in part to the inoculations.—Editors.]
MADURA FOOT

This disease is quite common in India and in various parts of the tropics; it has also been observed in Algiers and in America.

Madura foot, or mycetoma, resembles very closely actinomycosis in the general character of the lesions and the arrangement of the parasite in the form of colonies or granules. There seems to be but little doubt that these two conditions are distinct and that there are two varieties of Madura disease caused by different organisms. This disease usually assumes an extremely chronic character. The local disease is incurable except by operation. The organism causing this disease never develops secondary lesions in the internal organs. The most frequent part affected is the foot: hence the disease is spoken of as "Madura foot." In the affected part is noted a slow growth of granulation tissue, having an irregularly nodular character. In the center of these nodules there occurs a purulent softening, which is often followed by the formation of fistulous openings and ulcers. There is usually considerable enlargement and distortion of the part, frequently followed by necrosis of the bones. It is not unusual to find within the softened cavities and also in the spaces between the fibrous tissue small round bodies or granules resembling actinomycetes. These granules are yellowish or pinkish in color, resembling fish roe. The black granules are similar to gunpowder, and may by their conglomeration form nodules of considerable size. Owing to these two kinds of granules this disease has been divided into two varieties, which are known as the pale and black varieties. In both instances the granules are larger in size than in actinomycosis.

Morphology.—Morphologically this organism closely resembles actinomyces, but it possesses certain differences in cultural characters. It does not liquefy gelatin, but forms raised yellowish colonies, having umbilication of the center. On agar the growth is reddish in color. The organism grows well in the various vegetable infusions in which the actinomycetes does not grow. This organism grows only under aérobic conditions. Experimental inoculation of various animals has failed to reproduce the disease. There is no doubt that the black variety, or streptothrix madurae, and the actinomyces are distinct species.

Pale Variety.—When these roe-like granules are examined under the microscope they appear like actinomyces, showing an abundant mass of branching filaments with mycelial arrangement. The periphery of these masses sometimes shows club-like masses. These masses frequently are elongated and wedge-shaped, forming an outer zone to the colony which are connected by filaments.

Black Variety.—There has been considerable discussion in regard to the relation of this variety of the disease and the pale variety. Kan-
thack (4) believed that the organism was the same in both varieties, and occurred in the black variety only in a degenerated form. Boyce and Surveyor (2), on the other hand, have pointed out differences, showing that the black variety is more highly organized, and that the branching filaments are thicker, hence they believe that it belongs to the hyphomycetes. J. H. Wright’s (7) observations confirm this view. The pigment may be dissolved by treating the granules with hypochlorite of sodium solution. The granules are then crushed between cover-glasses and examined. The black granules are composed of a homogeneous substance impregnated with pigment; in this substance there are mycelium with thick filaments or hyphae and swollen segments, forming a zone with radiate arrangement. In the older granules the organisms are largely degenerated and present an amorphous appearance. Wright transferred a number of these black granules into various culture media, and was partially successful in growing the hyphomycete. The organism grows well on the ordinary culture media; on agar it forms a felted grayish mass; in old cultures the black granules appear with the mycelium. Microscopically, the organism appears as a mycelium having thick branching filaments with delicate transverse septa. In the older threads the segments are swollen, forming a string of oval-shaped bodies. So far as is known no spores are formed. Animal inoculations using cultures or the black granules from tissues have thus far been unsuccessful. Vincent (6) found that iodid of potassium, which has a high value as a therapeutic agent in many cases of actinomycosis, has no effect in the case of Madura diseases.

REFERENCES

1. Besson, A. Practical Bacteriology, Microbiology, and Serum Therapy, 572.
3. Israel. Neue Beobachtungen auf der Gebeite des Mykosen des Menschen, Virchow's Arch., lxxvi, 1870, s. 11.
4. Kanthack, A. A. Jour. Path. and Bacteriology, i, p. 140.
CHAPTER XVI

SPECIFIC PROPHYLAXIS AND THERAPY IN DIPHTHERIA

G. H. WEAVER

The history of the growth of knowledge of diphtheria is one of the most interesting topics in the whole field of medicine. Diphtheria as a distinct disease was first clearly described by Bretonneau in 1821, and by him and his pupils, including Velpeau and Trousseau, was developed a line of treatment to which but little was added until after the discovery of the diphtheria bacillus by Klebs in 1883 and its successful cultivation in pure culture by Loeffler in 1884.

The study of the diphtheria bacillus as to its effects upon the infected individual soon disclosed the fact that the bacilli remain almost entirely localized at the site of infection and exert their deleterious effects both locally and at distant parts of the body through poisons which they elaborate in their growth. These poisons or toxins are readily soluble and are taken up by the lymphatics from the local area of infection and finally reach the blood, thus being brought into contact with all the body structures.

In 1888 Roux and Yersin (24) demonstrated the presence of these toxins in the filtrate of an old bouillon culture of diphtheria bacilli. It then became possible to study the effects of the poisonous products of the diphtheria bacillus in varying doses without giving rise to a progressive infection by living bacilli. Thus it was shown that the cellular alterations in various structures of the body produced by the soluble toxins are identical with those which accompany an infection by living bacilli.

The first investigators to publicly announce the power of the blood serum of animals which had been artificially immunized with diphtheria toxins to protect and cure susceptible animals infected with living diphtheria bacilli or injected with their toxins were Behring and Wernicke (5) in August, 1891. In 1892 they described their experiments in detail and formulated the essential principles involved in the treatment of diphtheria by antitoxic serum. The injection of sublethal doses of diphtheria toxins in animals brought about an immunity to the living bacilli as well as to their toxins (active immunity). If the blood serum of such
PRODUCTION OF DIPHTHERIA ANTITOXIN

an immunized animal was injected into a susceptible animal, it was able to protect it against the subsequently inoculated diphtheria toxins or living diphtheria bacilli, or to cure it if the toxins or bacilli had been previously introduced (passive immunity). Near the end of 1891 Behring and Wernicke administered the antidiphtheria serum for the first time to a sick child in Berlin. They were followed by others, and the serum was soon being tested by clinicians throughout the world. The serum was used in man only after extensive tests had shown its undoubted value as a prophylactic and curative agent in lower animals. We quote from an early article by Welch (31) upon the treatment of diphtheria by antitoxin:

The discovery of the healing serum is entirely the result of laboratory work. It is an outcome of the studies of immunity. In no sense was the discovery an accidental one. Every step leading to it can be traced, and every step was taken with a definite purpose and to solve a definite problem. . . . These studies and the resulting discoveries mark an epoch in the history of medicine. It should be forcibly brought home to those whose philozoic sentiments outweigh sentiments of true philanthropy that these discoveries, which have led to the saving of untold thousands of human lives, have been gained by the sacrifice of the lives of thousands of animals, and by no possibility could have been made without experimentation upon animals.

Practical Production of Diphtheria Antitoxin.—The earlier studies on the action of the diphtheria bacillus and its toxins were accomplished through the use of small laboratory animals, and the first demonstrations of diphtheria antitoxin and its protective and curative power were also made possible by the utilization of these animals. The need for larger amounts of antidiphtheria serum led to the use of goats and sheep, and later of the cow and horse. For the production of the antitoxic serum on a large scale the horse has been found the most desirable animal for several reasons, and has come to be universally employed for the purpose. Healthy young horses are selected, their freedom from tuberculosis and glanders being assured by tests with tuberculin and mallein. Before and during treatment they are protected against infection by tetanus by injections at regular intervals of tetanus antitoxin.

The horse is first given subcutaneously a very small amount of diphtheria toxin, which is often combined with some antitoxin. The toxins are secured by growing diphtheria bacilli on the surface of alkaline broth, killing the bacilli by the addition of an antiseptic, and filtering. The fluid thus secured contains the diphtheria toxins in solution. The first injection in the horse is followed by some local swelling, a rise in temperature, and general indisposition. When the animal has recovered entirely from the effects of the injection a second is given, consisting of a slightly larger amount of toxin. The injections are given at intervals of a few days, the doses being gradually increased, until finally after several months an
enormous quantity can be borne with little inconvenience. After the horse has received injections of toxins for several weeks a sample of blood is drawn from the jugular vein with antiseptic precautions and the serum tested for antitoxin. When the amount of antitoxin has reached a sufficient height the horse is bled a larger amount. After each injection of toxin the amount of antitoxin in the blood falls and then rises until it is at its height about ten days after the injection. This time is chosen as the best for collecting the blood. After the horse is bled, an interval of a few days is allowed for recovery, and then injections of toxin are resumed. Not all horses produce antitoxin in large quantities. About 50 per cent. of young horses will yield 300-unit serum and about one-third 500-unit serum. A few horses, about three to six per cent., will yield as high as 1,000-unit serum. After the maximum of antitoxin production has been reached there is a decline, and few horses are useful as producers of antitoxin for more than a few months.

During the time that a horse is producing antitoxin in suitable amount it is bled at intervals of about four weeks. The amount of blood drawn at each bleeding is four, six, or eight liters, which is well borne by most horses. The blood is drawn from the jugular vein in the neck through a sterile trocar and tube, the hair having been removed, the skin sterilized, and the vessel being centrally compressed. If the whole serum is to be used for therapeutic purposes the blood is drawn into tall, narrow vessels and allowed to clot and the serum to separate. The serum is then drawn off and the proper proportion of some preservative added. In this country it is usual to add 0.3 per cent. of tricresol. To get rid of the precipitate which forms and to insure the sterility of the serum, it is passed through a porcelain filter and stored until needed. A sample is tested for its antitoxic content.

If the antitoxin is to be concentrated by the Gibson method enough sodium citrate (one per cent.) is added to the blood when drawn to prevent coagulation, and the plasma is drawn off after the blood corpuscles have settled out or have been thrown out by centrifugalization. The practical concentration of diphtheria antitoxin was first accomplished by the investigators in the Research Laboratory of the Department of Health of New York City. As first developed by Gibson (8) and later modified by himself and Banzhof (3), it has been extensively adopted by producers of diphtheria antitoxin in America. The following description of the process as at present employed is that given by Banzhof (2). Antitoxic plasma, in lots of fifty liters, is heated in a water bath at a temperature of 57° C. for from 12 to 15 hours, cooled down to room temperature and diluted with 25 liters of water. Saturated ammonium sulphate is added in the proportion of 3 c. c. saturated ammonium sulphate in 10 c. c. diluted plasma. The resulting fractional precipitate will contain all the euglobulin together with a small amount of antitoxin and pseudoglobulin.
This precipitate is taken up in saturated sodium chloride solution, filtered, and again precipitated by the addition of a half volume of saturated ammonium sulphate solution. The filtered precipitate is pressed dry with paper and dialyzed in parchment, which is continued for six to eight days. The filtrate from the first precipitation of the diluted plasma is measured and brought up to 54 saturation ammonium sulphate, according to the following proportions: 24 c. c. saturated ammonium sulphate in 10 c. c. filtrate. The resulting precipitate contains only the globulins soluble in saturated sodium chloride solution together with the antitoxin. This second precipitate is pressed to remove excess of ammonium sulphate solution containing the albumin and placed in dialyzing bags. Dialysis is continued until all traces of ammonium sulphate are removed, requiring six to eight days in running water. After dialysis is complete sufficient sodium chloride is added to bring the solution up to 0.08 per cent. It is customary to add 0.3 per cent. of tricresol to the finished product, after which the solution is rendered sterile by double filtration through Berkefeld filters. A sample is drawn off and tested for its antitoxic content, and the rest is stored in a refrigerator away from light until needed.

Standardization of Antidiphtheric Serum.—It will be of interest to briefly retrace the most important steps by which the present unit of diphtheria antitoxin has been reached. There is no chemical process by which the amount of antitoxin in a serum can be measured. All tests are physiological and are dependent upon the fact that guinea-pigs of about the same weight are very uniformly susceptible to diphtheria toxin. When diphtheria antitoxin was first used in the treatment of diphtheria in man, the amount of antitoxin was estimated in different ways by various producers so that there was no constant amount of antitoxin represented by a unit. In the first place the potency of a serum was measured by its ability to prevent infection by living cultures and later by its neutralizing power over diphtheria toxins. Roux and Behring first employed living cultures, but later substituted solutions of diphtheria toxins. In the method first employed by Roux the ratio between the amount of serum required to protect a guinea-pig against a fatal dose of living culture and the weight of the pig served to indicate the strength of the serum. When 0.01 c. c. of antitoxic serum was injected subcutaneously into a guinea-pig weighing 250 gm. and 12 hours later 0.5 c. c. of a fresh living culture was inoculated into the pig, if the guinea-pig lived the strength of the serum was said to be 1-25,000. In Behring's method a unit was such amount of serum as would protect a guinea-pig against 10 times the minimal fatal dose of the diphtheria toxin, and a normal serum was one of which 1 c. c. would just neutralize ten times the minimal fatal dose. The value of the serum was then indicated in normal units. In these earlier tests the antitoxin and toxin were injected at different places in the subcutaneous tissues of the test animal. In 1894 Ehrlich and Wassermann introduced the
method of testing in which the toxin and antitoxin are mixed outside the body and the mixture then injected into the guinea-pig. The toxin was considered neutralized if no local or general signs of intoxication appeared. In this method an antitoxic unit was such amount of serum as completely neutralizes ten times the minimal fatal dose of toxin. Still later Behring and Ehrlich further modified the unit and called an immunity unit such an amount of antitoxin as will neutralize 100 times a minimal lethal dose of toxin for guinea-pigs weighing 250 gm. In this case complete neutralization as shown by absence of local changes at the site of injection was insisted on. Further study showed that a partial neutralization of the poison, sufficient to save the animal, but not to prevent all local changes is more reliable. Up to this time all efforts to measure the strength of antitoxic serum were directed toward determining the quantity of serum required to prevent the effects of diphtheria bacilli or their toxins upon animals. Ehrlich now showed that, owing to variations and deteriorations in diphtheria toxins, it required varying amounts of antitoxin to neutralize the ten minimal lethal doses used in the test. Since a toxin, because of its unstable character, cannot be trusted as a standard, Ehrlich changed the standard of measurement from the toxin to the antitoxin. Antitoxin will remain unaltered if it is protected from heat, light, oxygen and moisture. Ehrlich took a carefully tested serum, reduced it to dryness, and preserved it in small portions in hermetically sealed tubes at a low temperature and away from light. At intervals one of these tubes is opened, the contents dissolved, and a definite amount of the resulting solution is said to contain a unit of antitoxin. This is the official standard for Germany, and it is an arbitrary amount of antitoxin, which Ehrlich believes to contain 200 combining units and is able to neutralize 200 minimal lethal doses of a pure toxin. The Ehrlich standard unit has been generally adopted. The standard antitoxic unit for the United States is established by the Hygienic Laboratory at Washington (22), and corresponds accurately to the Ehrlich unit. The Hygienic Laboratory at intervals of two months furnishes all licensed manufacturers of antitoxin with a solution of antitoxic serum, a certain amount of which contains one unit. With this as a standard all antidiptheric sera sold in interstate commerce must conform: The manner in which the amount of antitoxin in a serum is determined is as follows: A suitable toxin having been selected, it is carefully standardized and its L₄ dose determined. The L₄ dose is such an amount of diphtheria toxin that if mixed with one unit of antitoxin enough toxin will remain unneutralized to kill a guinea-pig weighing 250 gm. in four days. This dose having been determined, a series of guinea-pigs are injected with this amount of toxin plus various quantities of the serum under test. In this way is determined the smallest quantity of the serum required to protect a pig from death on the fourth day. This amount of serum is said to contain one unit, and from this
the number of units in each cubic centimeter of serum is readily calculated.

The method of Ehrlich for standardizing diphtheria antitoxin has been attacked by some investigators as not therapeutically accurate. The opposite view is held by Ehrlich and his followers and by most of those who have studied the subject. It has been claimed that antitoxic serums contain other immunizing and healing substances than antitoxin. Steinhardt and Banzhaf (29), after a careful experimental study, conclude that the therapeutic value of antidiphtheric serums depends on the number of antitoxic units present, and the antidiphtheric serum contains no protective substances aside from the antitoxin which play an important rôle therapeutically.

**Distribution, Keeping Qualities, and Government Regulation of the Manufacture of Diphtheria Antitoxin.**—The user of antitoxin is supplied with the serum in containers, each of which holds such a number of units as are suitable for single doses. By placing varying quantities in the packages offered the physician is able to select one which contains the number of units required by the individual case. It is customary to offer the serum in packages containing 1,000, 2,000, 3,000, 5,000, and 10,000 units. The serum may be placed in small bottles, closed with rubber stoppers or cork stoppers carefully paraffined or in glass ampules which are hermetically sealed. In the United States it has become the general custom to furnish the serum in a syringe package, so that the physician needs only to attach the needle before making the injection. This adds a little to the cost of the serum, but has the advantage of obviating the need of a syringe, which must be sterilized each time before being used. Each lot of serum, before it is put in the containers, beside being tested for its antitoxin content, is also tested for sterility by aërobic and anaërobic cultures, and for toxic substances, especially for bacterial poisons, by the injection of 5 c. c. into the peritoneum of a guinea-pig. The label on the package shows the number of units contained therein, also an expiration date beyond which it ought not to be used. It is customary to add an excess of 25 to 30 per cent. over that indicated on the label to allow for deterioration up to the date of expiration. The loss of antitoxin from age is much less than was at first supposed, provided the serum is sterile and the container properly sealed. Anderson (1) studied the influence of age and temperature on the potency of diphtheria antitoxin, and found that the yearly average loss in potency at room temperature is about twenty per cent. There was little difference between the keeping qualities of untreated sera and sera concentrated by the Gibson method. Anderson advised the addition of an excess of 33 per cent. to packages to allow for decrease in potency, and recommended that the date of expiration be not more than two years after the date of testing. Boehncke (6) has made an exhaustive study of the keeping qualities of diphtheria
antitoxin. He examined serums which had been kept in an ice-box without special precautions. He found no serum which showed loss of potency under four years, and some not under twelve years. A loss of ten per cent. occurred only after ten and fifteen years. It is evident that if serums are properly protected from heat and light they will preserve their potency for a very long time, and that serums which have passed their expiration date may still be used with confidence if they have been kept properly in the interval.

The Hygienic Laboratory of the Public Health and Marine Hospital Service at Washington exercises supervision over the production and sale of diphtheria antitoxin as related to interstate commerce. Producers of antitoxin operate under a license issued by the Treasury Department after an inspection has found the conditions of production satisfactory. The number of the license must appear upon the label of each package offered for sale. At intervals the stables, laboratories, and records of each manufacturer are officially inspected and samples of the various grades of antitoxin made by each licensed manufacturer are purchased in the open market and are tested in the Hygienic Laboratory for potency, freedom from contamination by foreign bacteria, and absence of chemical poisons, especially tetanus toxin.

SPECIFIC PROPHYLAXIS AGAINST INFECTION BY DIPHTHERIA BACILLI

Most of our efforts heretofore to protect individuals specifically against infection by diphtheria bacilli have been directed toward introducing preformed antitoxin, usually horse antitoxin, into the blood. The subeutaneous injection in man of a relatively small amount of antitoxin, 200 to 1,000 units, is followed by a very definite protection for a short time. This lasts only about three weeks. The foreign antitoxin is quickly eliminated and the original susceptibility returns. The use of immunizing doses of diphtheria antitoxin to protect persons, especially children, who are exposed to infection by diphtheria, was formerly used very commonly. In more recent times a considerable hesitancy in following this practice has been shown by many because of the many annoying and occasionally dangerous reactions liable to follow a primary injection, and because of hypersensitization to subsequent injections likely to follow the primary one. The danger from anaphylactic manifestations is, however, very slight if persons who are specially susceptible are excluded, namely, those affected with asthma. Since diphtheria is so readily controlled by antitoxin if seen at the very beginning, the prophylactic injection may be omitted in adults and children when they can be closely watched after exposure. In surroundings in which diphtheria is present, and in which.
conditions are unfavorable for the prompt recognition of diphtheria at
an early moment, all children should be protected by the administration
of from 500 to 1,000 units of antitoxin. The danger of producing a
hypersensitiveness to subsequent injections of antitoxin may be obviated
by using for prophylactic injections an antitoxin produced from some
other animal than the horse. The serums of cows and sheep have been
used and recommended for this purpose. In hospitals for contagious
diseases, where isolation cannot be carried out efficiently, the use of diph-
theria antitoxin as a prophylactic against secondary infection with diph-
theria in patients suffering from other contagious diseases has been em-
ployed with much advantage. This is especially advisable in cases of
scarlet fever and measles. The routine use of prophylactic injections of
diphtheria antitoxin in the inmates of institutions for children has been
found very helpful in some instances, but with proper isolation of in-
coming children and exclusion of bacillus carriers and other sources of
infection this would seem to be unnecessary. It is well known that certain
individuals do not contract diphtheria when exposed to the infection, and
that persons who have been in contact with cases of diphtheria may harbor
the bacilli in their throats for long periods without showing any signs of
acute infection, either through antitoxin in the blood or through an ac-
quired ability to produce antitoxin very promptly. Schick (25) has
devised a method by which the presence of diphtheria antitoxin in the
blood can be determined much more easily than by former methods. A
minute quantity of diphtheria toxin is injected into the skin and a local
reaction does not follow if diphtheria antitoxin exists in the blood, but
does occur if there is none. The amount of toxin injected is about
1/50 of a minimal lethal dose for a guinea-pig. The reaction occurs
after 24 to 48 hours, consists of local redness and infiltration, and sub-
sides, leaving pigmentation and slight scaling. A negative result always
indicates the presence of protective bodies in sufficient quantity for pro-
phylactic purposes. Positive results do not indicate with the same degree
of certainty the absence of protective bodies, since a few individuals show
an inflammatory reaction at the site of injection in spite of protective
bodies. Protective bodies are absent in fresh cases of diphtheria before
a serum injection. Schick's observations, together with those of other
observers, indicate that immunity to diphtheria exists at birth in about 80
per cent., reaches about 50 per cent. at one year of age, falls to 40 per
cent. at from two to five years of age, and rises to 90 per cent. in adults.
These figures indicate that prophylactic injections are unnecessary in
most infants and adults, and are specially indicated in children from two
to five years of age. By using Schick's test it may be possible to limit
immunizing injections of diphtheria antitoxin to those who require the
protection, and to avoid the procedure in those who are already protected.

In view of the disadvantages and occasional danger from using anti-
toxic serum for immunizing purposes, it would be desirable to have some other method of bringing about an immunity to diphtheria. There has been developed such a method by means of which an active immunity is produced which has the additional advantage over the passive in that it is more permanent.

In 1907 Theobald Smith (27) reported some experiments on guinea-pigs instituted to determine the degree and duration of passive immunity to diphtheria toxin transmitted by immunized female guinea-pigs to their immediate offspring. In a communication (28) in 1909 he added further observations in the same direction. He demonstrated that an active immunity lasting several years can be produced in guinea-pigs by the injection of toxin-antitoxin mixtures which have no recognizable harmful effect, either immediate or remote. Mixtures which produce local lesions and which, therefore, contain an excess of toxin, produce a much higher degree of immunity than the neutral mixtures, while an excess of antitoxin in the mixture reduces the possibility of producing an active immunity, and may extinguish it entirely. Smith's experiments prove that a relatively high degree of active immunity can be produced by a harmless procedure, while the use of toxin alone, leading to very severe local lesions, is incapable of producing more than an insignificant protection. He stated that the method invited further tests in regard to its ultimate applicability to the human being, but for this purpose the proportion of toxin and antitoxin which would produce the highest desirable immunity consistent with the least discomfort would have to be carefully worked out for the human subject. He also called attention to the obvious fact that such a method of immunization could not take the place of a large dose of antitoxin in exposed individuals who must be protected at once, and that it could only be useful as a general protective measure without reference to immediate danger, since it would take several weeks or longer to perfect the attainable immunity.

In Smith's experiments the presence of active immunity in the female guinea-pigs was determined by subjecting their offspring to injections of diphtheria toxins. He had shown that several litters of the offspring of female guinea-pigs, actively immunized to diphtheria toxin, inherit an immunity to diphtheria toxins. This is not the case if the mother has been passively immunized by the injection of diphtheria antitoxin alone, except when the injection is made during pregnancy and the antitoxin transmitted through the placenta. In this case the offspring is also passively immunized and the protection is of short duration. More recently Behring (4) has made further studies of the immunizing properties of mixtures of diphtheria toxins and antitoxins, and after extensive experimentation in lower animals has applied the method in man. Behring rests his protective vaccination upon the hypothesis that, with sufficient antitoxin in the blood, sickness from diphtheria is impossible. When a
child of 30 kg. receives 300 units of diphtheria antitoxin from the horse. The resulting antitoxin in the blood cannot be more than 1/10 of a unit per c. c. Clinically it is known that such an amount is usually protective against infection, and probably infection will be very exceptional if the blood contains 1/20 of a unit in each c. c. Antitoxin from the horse is rapidly eliminated from the human body. If 100 units are injected subcutaneously in a child a temporary immunity is produced lasting about ten days, but in twenty days the antitoxin has almost entirely disappeared, the blood containing less than 1/1000 unit per c. c.

If a homologous serum is injected the results are different. When human serum containing 300 units of antitoxin was injected into a child of 4 kg. the antitoxin in the blood soon stood at 1/5 of a unit per c. c., and then gradually fell to 1/20. Thirty-one days after the injection the blood still contained 1/25 of a unit per c. c., at which time horse serum antitoxin would have been entirely eliminated. Behring had previously made corresponding observations as to the slow elimination of homologous antitoxin from the blood of various domestic animals. Horses actively immunized against diphtheria toxins still contain considerable antitoxin in the blood several years after the treatment has been discontinued. The antitoxin here being homologous behaves like that which is introduced preformed. In man antitoxin produced actively under the influence of diphtheria toxin persists in the blood for a long time. Behring has made use of a modification of Römer's intracutaneous method in estimating the amount of antitoxin present in the blood of individuals. This consists in injecting into the skin of a guinea-pig mixtures of a standard dose of diphtheria toxin and various quantities of serum to be tested. The toxin having been previously standardized against a known amount of diphtheria antitoxin, the results as indicated by the local reaction serve to show the amount of antitoxin in the serum under test. In his immunizing procedure Behring injects his vaccine into or under the skin. The vaccine consists of a mixture of very strong diphtheria toxin and antitoxin in such proportion that when injected into guinea-pigs little or no toxic effects follow. The exact proportions have varied in different vaccines employed. The immunizing effects from the intracutaneous and subcutaneous injections are about equal, but the reaction from the intracutaneous injection is less than that from the subcutaneous. It is desirable to give the first injections intracutaneously, as the resulting reaction can be better observed. In any case a local reaction seems essential in order that antitoxin may be produced. Zangemeister (32) found newborn infants very resistant. They bear about one hundred times as much of the vaccine in proportion to weight as do adults. In animals, as well as man, it has been shown that the difference between the dose of vaccine which causes a barely recognizable reaction and a severe but not dangerous one is very considerable. The immunizing results of injections of the
toxin-antitoxin mixture have been determined by subsequent tests of the blood for antitoxin. In one exceptional instance Matthes (14) found that the total production of antitoxin in the blood from a single injection was more than 600,000 units. Kleinschmidt and Viereck (12) consider 1/20 of a unit of antitoxin in each centimeter of blood to be sufficient to protect the individual against severe epidemic infection. To secure this they advise two injections in most cases, especially if the second has been followed by local redness and infiltration over 2 cm. in diameter with pronounced spontaneous pain or tenderness. Out of forty cases receiving the injections in only five was there any elevation of temperature, and in only two any enlargement and tenderness of lymph glands. The injections were entirely harmless, usually causing but slight local irritation for three or four days. When more than one injection was given the interval was usually from three to five days. The immunizing effects were usually demonstrable in the blood by the detection of antitoxin 23 to 25 days after the injection; in only one instance as early as the 21st day. The amount of antitoxin in the blood was usually greater in proportion to the number of injections. Out of 21 cases receiving 2 injections the lowest resulting antitoxin production was 0.075 unit in 1 c. c. of blood, or about 187.5 units in the total blood; the highest was 1 unit per c. c. of blood, or about 2,500 units as a whole. In 4 of the 21 cases no increase of antitoxin was found. In 10 patients receiving many injections of small, gradually increasing doses the antitoxin content of each centimeter of blood rose to 0.5 to 1.0 unit, or a total of 250 to 2,500 units. Nine patients received 4 to 7 injections of rapidly increasing doses, and in these the antitoxin content of the blood per c. c. rose to 10 to 75, or reached a total of 25,000 to 187,500 units.

Kissling (10) has vaccinated 310 persons exposed to infection by diphtheria with the Behring toxin-antitoxin mixture. Most of these were children, and many were cases of scarlet fever, measles, and other contagious diseases. In the first 199 cases one dose only was given, and in the remainder two doses. The injections were made partly subcutaneously and partly intracutaneously, and were made in the back in the interscapular region. Of the 199 persons who were vaccinated once 8 contracted diphtheria, and of the 111 persons, equally exposed to infection, who were vaccinated twice, none contracted the disease. The disturbances from the vaccinations were no greater than those following vaccination for smallpox, and the vaccinations were entirely harmless.

The procedure devised and advocated by Behring is still in the experimental stage, and further use in the hands of those familiar with the technique is required before it can be safely introduced into general use. The exact dosage of the mixture employed, and the accurate standardization of the vaccinating mixture, must be further determined. Resting as it does on solid experimental foundation, it is not too much to hope that
TREATMENT OF DIPHTHERIA

Since the introduction of diphtheria antitoxin in the treatment of diphtheria this remedy has displaced all measures formerly employed. The results of the use of antitoxin were shown at once in a pronounced reduction in mortality, and subsequent results have been even more striking, owing to the prompt use of larger doses. There can be no doubt that a still further reduction in the mortality from diphtheria of no inconsiderable degree might be effected if all cases were brought under treatment early in the disease and were given efficient doses of antitoxin. Experiments upon animals show that a given quantity of antitoxin will neutralize only a certain amount of toxin. This is the practical conclusion of all observers, although there is some difference of opinion as to the exact chemistry of the reaction. The purpose of treatment is to give enough antitoxin to neutralize the toxins in the body of the infected individual at the earliest moment before permanent and irreparable damage has been done to muscular, glandular, and nervous structures. The antitoxin cannot undo this damage, but can only prevent further injury, and if the changes already produced by the toxins before the antitoxin has been administered are not such as to cause death nature may repair the damage. It follows that the vital element in the use of diphtheria antitoxin is its early administration in sufficient doses.

We have no method of measuring the amount of toxin already produced in the infected individual. The longer the case has been in progress, and the more extensive the surface involved, the greater the intoxication. The virulence of the infecting bacilli is also important, and this may be judged from the severity of the general toxic symptoms. The dose of antitoxin to be employed in various cases is to be determined by consideration of the factors mentioned. Cases presenting the clinical picture of diphtheria should receive the antitoxin at once, even before a bacteriologic diagnosis has been made. Only in very mild and early cases with a doubtful diagnosis is it justifiable to wait for the growth of cultures. In more severe cases, with much decomposing secretions, a negative culture must not be allowed to interfere with promptness and persistence in the treatment. Some general guide to dosage may be given, but this must be varied in accord with the judgment of the physician in the individual case. When the case is in the first day, and the exudate limited to one tonsil, the proper dose would be 2,000 to 3,000 units, if both tonsils are involved 5,000 units. If the case has been running two or three days the dose should be double that just indicated. When the membrane has ex-
tended beyond the tonsils to the soft palate, pharynx, nasopharynx or nose, and where the cervical glandular and periglandular swelling is extensive, the dose should be 10,000 to 20,000 units, according to the degree of intoxication. In cases of laryngeal diphtheria in which there is no distinct and permanent obstruction 8,000 to 10,000 units would be a suitable dose, but when the symptoms are more severe and signs of obstruction of the larynx are becoming more pronounced 20,000 are to be given at once. In all cases it is desirable to give at the primary injection as near as possible to the total amount which the case will require. The need of injections subsequent to the primary one and the doses to be employed are to be determined by the effects of the primary dose. When doses such as indicated above have been followed by no improvement in 24 hours the injection is to be repeated, always in as large a dose as at first, and if the disease has progressed in the interval the second dose may be larger than the first. In very sick persons with extensive membrane and severe intoxication the second injection may be given in twelve hours. Later injections are to be determined by the same criteria as the second.

The nephritis which occurs in the early stage of diphtheria appears to be favorably influenced by antitoxin. It has sometimes been stated that postdiphtheritic paralysis occurs more frequently since antitoxin has been used than formerly. Doubtless some severe cases of diphtheria which come under treatment relatively late are rescued from acute death by antitoxin, and subsequently develop late paralysis. This is due to changes in the nervous structures which have occurred before the antitoxin has neutralized the toxin. Rosenau and Anderson (23) made a careful study of the influence of antitoxin upon postdiphtheritic paralysis in guinea-pigs, in which animals the conditions correspond very closely with those in man. They found that antitoxin given 24 hours after the infection can save the life of the guinea-pig and greatly modify the paralysis, but that a single large dose 48 hours after the infection did not modify the paralysis or save life. Repeated injections beginning 24 or 48 hours following infection seemed to have a more favorable effect upon the subsequent paralysis than a single injection. Antitoxin had no influence in preventing paralysis if injected shortly before the paralysis developed, and had no influence upon the diphtheritic paralysis after it had appeared. A very small amount of antitoxin (in a guinea-pig—1 unit) given 24 hours before or at the time of infection prevented the development of paralysis. They point out that in this experimental work upon guinea-pigs an early and malignant form of experimental postdiphtheritic paralysis is being dealt with, which grave variety is rare in man, and that the entire charge of poison is injected directly into the tissues of the guinea-pig, while in man the toxin is doubtless elaborated more slowly. It is therefore likely that antitoxin given in man at a somewhat later period than in guinea-pigs would exert beneficial effects.
In a recent experimental study of diphtheritic paralysis in which guinea-pigs were employed Römer and Vierkeck (21) have reached results which correspond very closely with those just given. They conclude that diphtheria antitoxin in no way favors the occurrence of diphtheria paralysis, but may convert a case from one with acute poisoning to one with paralysis; that prophylactically given diphtheria antitoxin in very small amounts prevents every paralysis, and therapeutically given up to 24 hours after the poisoning it prevents or ameliorates the paralysis. They also found that a curative effect from diphtheria antitoxin in well-developed paralysis cannot be shown. Rolleston (20), from an extensive clinical experience, has reached the opinion that early injection of antitoxin undoubtedly jugulates the disease and tends to diminish the occurrence of subsequent complications. American physicians have been special advocates of large doses of antitoxin. McCollom (16), of Boston, was one of the first to urge the use of large doses of serum, and he bases his argument upon the more favorable results he secured with larger doses.

Since the changes in the nervous structure which are responsible for much of the fatality in diphtheria are only prevented by the early neutralization of the toxins in the circulation, it is very important that the antitoxin be administered in such a manner as to favor its rapid absorption.

Park (17), of New York, has made some valuable studies on the absorption of antitoxin when given subcutaneously and intravenously. After subcutaneous injection the antitoxin content of the blood increases rapidly in amount from the first to the twenty-fourth hour, and then, as a rule, more slowly for the next twenty-four hours to forty-eight hours, and in no case did the antitoxin content of the blood decrease until after the third day. When the serum is given intravenously the whole effect is added to the blood immediately, and during the first twenty-four hours averages more than five times as much as in cases in which the serum is given subcutaneously. The antitoxin content then falls steadily, but on the fourth day about one-half of the antitoxic strength still remains. As a result of his studies Park concludes that the first dose should be sufficient for the case, and that further doses should only be given, because one has not judged correctly the amount required in the first dose; and that, while there is no harm in giving additional doses, even if the first is sufficient, there is great harm in relying on later doses to add to the effect of the first. He advises in severe toxic cases 20,000 units in adults and 10,000 to 20,000 units in children. A repetition of the dose is unnecessary during the first forty-eight hours and seldom at a later time. In these cases the antitoxin should be given intravenously if possible. Moderate cases receive a single dose of 10,000 units, which is usually given subcutaneously, although an intravenous injection will produce quicker results. In mild cases 3,000 to 5,000 units are given.

When injected into muscles the antitoxin is absorbed much more
rapidly than when placed beneath the skin, but naturally does not act so quickly as when injected directly into a vein. Kleiner and Meltzer (11) have shown that substances, whether colloidal or colloidal, producing definite effects upon the animal body manifest these effects earlier and with greater intensity when administered by intramuscular than by subcutaneous injection. Morgenroth and Levy (15) found that the highest antitoxin value naturally occurs after intravenous injection, yet after eight hours the concentration appears to fall, while with intramuscular injection after eight hours the antitoxin content of the blood is nearly as great as after intravenous injections. In four to five hours the antitoxin content is five to twenty times, in seven to eight hours it is at least three to ten times as much after intramuscular as after subcutaneous injection, and after twenty-four hours it is still five times as great.

Römer and Viereck (21) summarize the indications for the administration of diphtheria antitoxin as follows: (1) earliest possible time; (2) if possible into veins, next into muscles, then subcutaneously; (3) sufficient quantity, but not unnecessary amount.

It is hardly necessary to state that all injections are to be given with strict aseptic precautions. The skin at the point of puncture is to be surgically clean, and the punctures to be sealed by an occlusion dressing, preferably held in place with collodion. Intravenous injections can usually be made by puncturing a superficial vein at the bend of the elbow when it has been distented by constriction of the arm. The injection should be made very slowly. For intravenous injections it is desirable if possible to use a serum which contains no antiseptic. The advantage of the antitoxic globulin solution prepared according to Gibson’s method is that a certain dose of antitoxin can be given in a smaller bulk and with less non-antitoxic proteins than in the whole serum. Park and Throne (18) conclude that the removal of a considerable portion of the non-antitoxic globulins, as well as of the albumins from the serum by the Gibson method, has eliminated much of the deleterious matter from the serum, so that severe rashes, joint complications, fever, and other constitutional disturbances are less likely to occur from the antitoxic globulins than from the antitoxic serum from which they were obtained.

Steinhardt and Banzhaf (29) conclude from studies upon guinea-pigs that the therapeutic value of the plasma is not appreciably impaired through the process of eliminating the albumins and other non-antitoxic proteins by the salting-out method employed and the final dialyization of the concentrated product.

As the local disturbances which accompany diphtheria subside the bacilli usually decrease in number, and in a considerable proportion of cases no bacilli are demonstrable by the time all membrane has disappeared. In other cases the bacilli remain in the throat for a longer or shorter time, and the person, being in good health, becomes a bacillus carrier. The
exact mechanism by which the body rids itself of the diphtheria bacilli is imperfectly understood. In order to shorten the period of quarantine and lessen the danger of spread of diphtheria efforts to get rid of the bacilli in the throat have been repeatedly made. After various antiseptic washes had been mainly used certain measures were introduced which call into play vital processes. In 1909 Schötz (26) directed attention to an apparent antagonism between the diphtheria bacilli and staphylococci in the throat. He noted that when the throat of bacillus carriers became infected with staphylococci the bacilli disappeared. He concluded that the cocci in some way killed out the bacilli. In imitation of nature he inoculated the throats of bacillus carriers with staphylococcus cultures and gave favorable reports therefrom. Following this lead, a considerable number of reports have emanated from persons who have used sprays of staphylococcus pyogenes aureus broth cultures with the object of eradicating diphtheria bacilli from throats and noses in which they had persisted for some time. Some reports have been favorable and some have been entirely negative. The usual application of the treatment has consisted in spraying a living broth culture of the staphylococcus pyogenes aureus into the nose and throat once or several times a day. Lydia M. DeWitt (7) studied the subject experimentally and found that on artificial culture media there is no inherent antagonism or incompatibility between staphylococcus aureus and the diphtheria bacillus. She concludes that the apparently favorable action of the staphylococcus aureus on chronic diphtheria cases seems to be an effort to reinforce the favorable, friendly throat flora in cases in which they are unable to regain their natural, normal ascendancy. Some severe infections by the staphylococci introduced in these sprays have been observed. In estimating the value to be attached to this treatment it must be remembered that the diphtheria bacilli in most cases disappear from the throat in a comparatively short time, and that persistent carriers are uncommon. No observations on a sufficiently large scale and adequately controlled have been reported to convince us of the value of the procedure, and the danger of causing an infection by the abundant staphylococci introduced must not be overlooked.

Efforts have also been made to get rid of the persistent bacilli by stimulating the forces by which the body rids itself of them. Diphtheria antitoxin administered subcutaneously, or applied locally, has no appreciable effect. Wassermann (30) and Lipstein (18) produced antitoxic sera by injecting the animal to be immunized with diphtheria bacilli or their extracts instead of with toxin. In this way they secured sera which possessed agglutinating power over diphtheria bacilli. It was hoped that such sera might be useful in ridding the body of bacilli, but the results of its local and general use were disappointing. More recently Hewlett and Naukivell (9) prepared a solution of the endotoxin of the
diphtheria bacillus by employing Macfadyan's method of grinding in the presence of intense cold and filtration. With this as a vaccine they claim to rid the throat of diphtheria bacilli. Petruschky (19) has employed a vaccine consisting of diphtheria bacilli devitalized by chloroform vapor. By injections of a suspension of these dead bacilli he produces an active immunity and believes that he gets rid of the bacilli which persist in carriers. In both of these modes of treatment by vaccines the end sought is to stimulate the body to produce antibacterial substances. Such bodies are, however, at a great disadvantage, since the offending bacilli are upon the surface of mucous membranes, where they are not readily reached by the immune bodies.

Kaolin has the faculty of mechanically removing bacilli from the surfaces of mucous membranes and has been used locally with success by Hektorn and Rappaport. Careful examination of persistent carriers often discloses local conditions which favor the retention of the bacilli, such as enlarged tonsils and adenoids, and sinusitis. The removal of such tonsils and adenoids and the employment of measures to secure free drainage of the accessory sinuses are often followed by prompt disappearance of the bacilli.

REFERENCES


REFERENCES


18. —— and Throne. The Results of the Use of Refined Diphtheria Antitoxin, Gibson’s “Globulin Preparation” in the Treatment of Diphtheria. Collected Studies from the Research Laboratory, Dept. of Health, City of New York, 1906, ii.


29. Steinhardt and Banzhaf. The Relative Therapeutic Value of Antitoxic Globulin Solution and the Whole Serum from Which It Is Derived. Collected Studies from the Research Laboratory, Dept. of Health, City of New York, 1907, iii, 150.
CHAPTER XVII
TETANUS

WILLIAM H. PARK

The treatment of tetanus has two distinct purposes: (1) the neutralization of the tetanus toxins, and (2) the sustaining of the patient and the alleviation of the symptoms until the effects of the specific poison subside, as shown in the relaxation of the muscular contractions. This article considers only the first purpose, as the general treatment and the detailed histories of individual cases are considered in Volume II, beginning at page 341.

The Bacterial Poisons.—The characteristic symptoms of tetanus are caused almost wholly by a very powerful poison produced by the tetanus bacilli. This is called tetanospasmin. This poison is given off by the bacillus and is of such toxic powers that 0.000,005 gm. will kill a mouse. There is a second poison elaborated by the bacilli called tetanolysin, which has the power to cause lysis of the red blood cells. This is less in amount and less toxic. Some consider it as a factor in the anemia occurring, but it probably has little deleterious effect. The endotoxins in the protoplasm of the tetanus bacilli are of no importance, since the tetanus bacilli develop only in small numbers, and long before the endotoxins could accumulate in appreciable amounts the more powerful tetanospasmin would cause death. The tetanus bacilli remain almost wholly at the site of the wound, a few only are carried to the blood and scattered throughout the body. These isolated bacilli apparently do not proliferate.

The Source of the Infecting Tetanus Bacilli.—It is a peculiar fact that these bacilli live and multiply in the intestinal contents of horses, cattle, dogs, and even men without causing injury. Unless the mucous membrane is wounded neither the tetanus bacilli nor their toxins are absorbed. The feces scatter the bacilli and their very resistant spores over the soil. These, consumed with the grass or inhaled with the dust and caught on the nasopharyngeal mucous membrane, enter the intestines of other animals and men.

As a rule, the warmer the climate the greater the proportion of animals and men with tetanus-infected feces. Certain localities are known to be
especially liable to tetanus infection, such as Eastern New York and Connecticut. The spores are very resistant, living almost indefinitely when protected from sunlight and moisture.

The Means by Which Wound Infection Occurs.—The tetanus bacilli and spores unaccompanied by other bacteria do not develop readily if located in healthy tissues. If, however, the tissues are injured, or they are accompanied by other bacteria or by foreign materials, the tetanus spores then develop and multiply and poisoning occurs. This is especially liable to take place in a ragged penetrating wound where the tissues adjacent to the infection are somewhat lacerated. The presence of a foreign body such as catgut, the waste from a blank cartridge, shreds of clothing, or simply dirt add to the danger. The additions to the foreign material of a few pathogenic or putrefactive bacteria adds still further to the probability of infection. If the wound is quickly and thoroughly cleaned infection is usually avoided, but if it is neglected, or if because of its nature it cannot be cleaned, tetanus may develop.

The Preventive Treatment.—This consists in the treatment of the wound and in the giving of antitoxin. The surgical treatment has for its object the removal of all foreign material including bacteria from the wound in so far as that is possible.

The surrounding parts should be thoroughly cleaned with soap and water, and the wounded tissues cleansed with sterile salt solution. All dirt, bits of clothing, and any foreign material should be carefully removed. Finally a thorough cleansing with some suitable disinfectant solution should be carried out. If the danger of tetanus or other bacterial infection is great pack the wound lightly with antiseptic gauze. Inject in all suspected cases from 1,000 to 2,000 units of tetanus antitoxin subcutaneously. The smaller dose is sufficient for young children, and in adults having but slight wounds. The antitoxin is eliminated at the end of two weeks. It is therefore wise to repeat the injection at the end of ten days in all cases where the wound is extensive or sloughing of tissues occurs. In these cases tetanus toxin may continue to be elaborated and absorbed.

Diagnosis.—This is generally made through the symptoms, and there is usually no need of a bacteriological examination before treatment is instituted.

The first suspicious symptoms should be the signal for immediate injection. If the case is one of tetanus the symptoms will develop in spite of this sufficiently to make the diagnosis certain. Bacteriological tests may be valuable in doubtful cases in confirming the diagnosis or in disproving it.

An infant, for instance, was reported as having developed fatal tetanus after vaccination. The skin and subcutaneous tissues were excised at the point of vaccination and placed in broth under anaerobic conditions. The
absence of the development of tetanus bacilli, together with the discovery at autopsy of an intense gastritis, eliminated the diagnosis of tetanus.

The Paths by Which Tetanus Toxin Reaches the Central Nervous System.—It is a matter of great practical importance to discover the course of the toxin from the wound to the cells of the brain and spinal cord, because our methods of injecting the antitoxin will be greatly influenced by the location of the toxin in the tissues at the time symptoms develop. Much experimental work has been done in investigating this subject. All agree that the toxin is taken up to some extent by the nerves. Some believe that this is wholly through the end nerve plates, and that the toxin passes along the nerve fibers until it reaches the spinal cord. Others think that the toxin passes up the lymph vessels of the nerves. There can be no doubt that a considerable amount of the toxin passes up the nerve trunk, supplying the region of the infection, but probably much the larger part is taken up by the tissue lymph spaces and carried through the lymph channels to the blood current, and there distributed through the body to pass out from the blood capillaries and be, if not already neutralized, taken up by the nerve endings everywhere throughout the whole body. The most important investigations upon this point may be summed up briefly as follows:

Gamprecht and Stintzing concluded from their experiments that the toxin from the wound passed to the central nervous system partly directly by the peri- and endoneural lymph spaces of the nerves of the infected region, which directly connected with the subdural spaces, and partly through other nerves obtaining it indirectly from the blood. The local tetanus they considered as due to the contact of the poison with the motor end plates.

The experiments of Meyer and Ransom and of Marie and Morax proved to their satisfaction that the poison is transported to the central nervous system by the way of the motor nerves—and by no other channel. These authors thought that they had shown that the essential element for the absorption and transportation of the toxin is not the lymph channels, but the axis-cylinder, the intramuscular endings of which the toxin penetrates. Marie and Morax were able to demonstrate the poison in the nerve corresponding to the area of infection one and one half hours after treatment. Absorption, however, and conduction are dependent to a large extent on the nerves being intact. A nerve cut across takes very much longer to take up the poison (about twenty-four hours), and a degenerated nerve takes up no poison whatever. In other words, section of the nerve prevents the absorption of the poison by way of the nerve channels. Similarly section of the spinal cord prevents the poison from ascending to the brain. The poison which passed through the general lymph channels to the blood was partly returned to the tissue fluids throughout the body and taken up by the nerve endings and thus pro-
duced general tetanus. According to Meyer and Ransom, the reason sensory nerves do not play any rôle in the conduction of the poison is because the spinal ganglion places a bar to the advance of the poison.

Ascending centripetally along the motor paths, it reaches the motor spinal ganglia on the side of inoculation and affects the ganglia of the opposite side, making them hypersensitive. The visible result is the highly increased muscle tonus, i.e., rigidity. If the supply continues the toxin next affects the nearest sensory apparatus; there is an increase in the reflexes, but only when the affected portion is irritated. In the further course of the poisoning the toxin as it ascends continues to affect more and more motor centers, and also the neighboring sensory apparatus leading to spasm of all the striated muscles and general tetanus.

Field has shown that not only tetanus toxin, but diphtheria toxin and inert colloids, can be demonstrated in the sciatic nerves after they have been injected subcutaneously or intramuscularly, and after varying periods may be found in the spinal cord. He believes that the toxin passing up nerve trunks is absorbed mostly by way of the lymphatics of the nerves.

Cernovodeanu and Henni confirm this contention. They ligated all the muscles and blood vessels in a guinea-pig’s leg, leaving intact only the sciatic nerve, skin, and bone, and then injected a large amount of tetanus toxin below the point of ligation. The animals never developed tetanus. There was only a very slight flow of lymph into the ligated area, and therefore only a slight flow up the nerve.

The larger part of the toxin is carried by the lymph of the infected region to the blood, and if not neutralized is transmitted to the tissue fluids. The path of absorption to the central nervous system is then by way of the motor nerve tracts of the whole body.

The Union of Toxin with the Gray Matter of the Brain and Spinal Cord.—This union is a loose one, and the toxin can be partially freed from its union by the action of proteolytic ferments. A number of different elements of the cell substance seem to have this power of binding the toxin. Heating to 65° C. for ten minutes destroys the capacity to fix toxin. These brain substances which unite with toxin are certainly not of the nature of antitoxin, and the brain cells, if they produce antitoxin at all, certainly share the power with other cells. Marie notes that adrenalin neutralizes tetanus toxin, and that lecithin compounds are concerned in the mechanism of the action of tetanus toxin on nerve cells.

Period Between Absorption of Toxin and Development of Symptoms.—There is, however, apparently an interval of time in which the toxin is in contact with the cells’ surface, or is free in the cells’ fluid, before true union takes place. According to experiments by Kraus, part of this toxin will pass out of the cells if they are surrounded by an antitoxic fluid, just as salts pass through a membrane into salt-free fluids. After the absorption of the poison there is a lapse of time before any
effects are noticed. With the injection of an enormous amount, such as 90,000 fatal doses, there is about nine hours; with 30,000, ten; with 3,000, twelve; with 10 fatal doses, fifteen to eighteen; with 2 fatal doses, fifteen to twenty-four. Less than a fatal dose will produce local symptoms in forty-eight to seventy hours. When living cultures are injected longer periods elapse, for then the toxins require time for production.

Muscles Involved.—The parts first to be affected with tetanus are, in about one-third of the cases in man, and usually in animals, the muscles lying in the vicinity of the inoculation—for instance, the hind foot of a mouse inoculated on that leg is first affected, then the tail, the other foot, the back and chest muscles on both sides, and the forelegs, until finally there is a general tetanus of the entire body. In mild cases, or when a dose too small to be fatal has been received, the tetanic spasm may remain confined to the muscles adjacent to the point of inoculation or infection. The symptoms following a fatal dose of toxin vary greatly with the method of injection. Intraperitoneal injection is followed by symptoms which can hardly be distinguished from those due to many other poisons. In man the first symptoms are usually those of a contraction of the muscles of the lower jaw, and then those of the neck.

Presence of Tetanus Toxin in the Blood.—The blood during the first four days of the disease usually contains toxin. After that time antitoxin usually develops and soon makes the blood antitoxic. In St. Louis some years ago the serum of a horse dying of tetanus was given by accident in doses of 5 to 10 c. c. to a number of children, with the development in some of fatal tetanus. In this connection Bolton and Fisch showed by a series of experiments that considerable toxin might accumulate in the blood before symptoms became marked. In the cases of human tetanus examined the amount of toxin present in the blood has not been large.

Endotoxins.—These are so much less poisonous than the tetanospasmin that they do not have any appreciable influence on the development of disease.

Specific Treatment of Tetanus

The Protective Action of Tetanus Antitoxin.—Behring and Kitasato were the first to show the protective and curative effects of the blood serum of immunized animals. It was found that animals could be protected from tetanus infection by the previous or simultaneous injection of tetanus antitoxin, provided that such antitoxic serum was obtained from a thoroughly immunized animal. This neutralization was due to a chemical union between the two substances. From this it was assumed that the same result could be produced in natural tetanus in man. Unfortunately, however, the conditions in the natural disease are very much
TETANUS

less favorable, inasmuch as treatment is usually commenced, not shortly after the infection has taken place, but hours after the tetanic symptoms have appeared when the poison has already attacked the cells of the central nervous system and to some degree permanently combined with them.

The Production of Tetanus Antitoxin for Therapeutic Purposes.—The tetanus antitoxin is developed in the same manner as the diphtheria antitoxin—by inoculating the tetanus toxin in increasing doses into horses. The horses receive 5 c. c. as the initial dose of toxin, of which 1 c. c. kills 250,000 gm. of guinea-pig, and along with this twice the amount of antitoxin required to neutralize it. In five days this dose is doubled. This over-neutralized toxin stimulates the production of antitoxin. Recently we have preferred to inject the horses subcutaneously with 5,000 units of tetanus antitoxin, and then, after a lapse of twenty-four hours, give, at short intervals, increasing doses of straight toxin. After four or five months of this treatment the blood of the horse contains the antitoxin in sufficient amount for therapeutic use. Horses usually have about 100 units, but some have produced as high as 600 units per c. c. The antitoxic serum is refined by eliminating all substances except the pseudoglobulins. As in the case of diphtheria antitoxin the tetanus antitoxin is bound with the pseudoglobulins of the horse serum.

Antitoxic Unit and Technique of Testing Antitoxic Serum.—Tetanus antitoxin is tested exactly in the same manner as diphtheria antitoxin, except that the size of the unit is different. In 1907 the producers of serum in the United States agreed to a unit of antitoxin which is approximately ten times the size of the unit of diphtheria antitoxin. A unit is defined as the amount of antitoxin required to just neutralize 1,000 minimal fatal doses of tetanus toxin for a 350-gram guinea-pig. The United States government has adopted this unit, and supplies the different producers for testing purposes with standardized toxin.

Antitoxic Units Adopted by Foreign Governments.—The amount of antitoxic serum which neutralizes an amount of test toxin which would destroy 40,000,000 gm. of mouse contains 1 unit of antitoxin by the German standard. In the French method the amount of antitoxin which is required to protect a mouse from a dose of toxin sufficient to kill in four days is determined, and the strength of the antitoxin is stated by determining the amount of serum required to protect 1 gm. of animal. If 0.001 c. c. protected a 10-gram mouse the strength of that serum would be 1:10,000.

Persistence of Antitoxic in the Blood.—Ransom has clearly shown that the tetanus antitoxin, whether directly injected or whether produced in the body, is eliminated equally slowly from the blood of an animal, provided that the serum was from an animal of the same species. If from a different species it is much more quickly eliminated and has practically disappeared in from ten to twenty-one days.
Absorption of Toxin and of Antitoxin from the Tissues.—The same investigator made very extensive and interesting observations on the absorption of the tetanus poison by the lymph vessels and its accumulation in the blood; he also made similar observations on antitoxin. He inserted in the thoracic duct of a dog a cannula and then injected in the subcutaneous tissues of the left inguinal region a large number of fatal doses of tetanus toxin. In another dog he performed the same experiment, except that he substituted antitoxin for toxin. He took samples of the lymph every few minutes after giving the injections, and measured the amount of toxin or antitoxin, as the case might be. He also made an experiment in which, some hours after the toxin had been administered, he later administered the antitoxin in another part of the body, and noted the time at which the toxic lymph became neutralized and then antitoxic. The following two tables show the results of the injection of the toxin and of the antitoxin:

**Table I**  
*Absorption of Toxin in Dogs as Shown in Lymph and Blood*  

<table>
<thead>
<tr>
<th>Lapse of Time after Injection</th>
<th>Fatal Toxin Doses for a Mouse in 1 c.c.</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 to 30 minutes</td>
<td>0</td>
</tr>
<tr>
<td>1 hour to 1 1/2 hours</td>
<td>10</td>
</tr>
<tr>
<td>2 hours to 2 1/2 hours</td>
<td>100</td>
</tr>
<tr>
<td>3 hours to 3 1/2 hours</td>
<td>200</td>
</tr>
<tr>
<td>4 hours to 4 1/2 hours</td>
<td>500</td>
</tr>
<tr>
<td>5 hours to 5 1/2 hours</td>
<td>1,280</td>
</tr>
</tbody>
</table>

**Blood**

<table>
<thead>
<tr>
<th>Lapse of Time</th>
<th>Fatal Toxin Doses for a Mouse in 1 c.c.</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 minutes</td>
<td>0</td>
</tr>
<tr>
<td>1 hour</td>
<td>5</td>
</tr>
<tr>
<td>4 hours</td>
<td>25</td>
</tr>
<tr>
<td>6 hours</td>
<td>35</td>
</tr>
</tbody>
</table>

**Table II**  
*Absorption of Antitoxin*  

<table>
<thead>
<tr>
<th>Lapse of Time</th>
<th>Number of Mouse Units of Antitoxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 15 minutes</td>
<td>trace</td>
</tr>
<tr>
<td>15 to 30 minutes</td>
<td>50</td>
</tr>
<tr>
<td>1 hour to 1 1/2 hours</td>
<td>6,000</td>
</tr>
<tr>
<td>2 hours to 2 1/2 hours</td>
<td>25,000</td>
</tr>
<tr>
<td>3 hours to 3 1/2 hours</td>
<td>55,000</td>
</tr>
<tr>
<td>4 hours to 4 1/2 hours</td>
<td>100,000</td>
</tr>
</tbody>
</table>
It is noticed that in the above tables the lymph remained up to 30 minutes free of toxin. It then began to appear in increasing amounts up to the end of the experiment at five hours. The blood remained free from toxicity as long as the lymph and then to a lesser degree, so that there is no question but that the blood vessels themselves did not take up any appreciable tetanus toxin except as it was delivered to the blood stream by the lymph. In the second experiment in which the antitoxin was injected it is noticed that even at 15 minutes a trace of antitoxin appeared in the lymph. This rapidly increased until the end of the experiment at 4½ hours. Here, again, the blood stream accumulated antitoxin only as it was poured in by the lymph. In a third experiment an intravenous injection of antitoxin was given. In a very few minutes the lymph showed distinct amounts of tetanus antitoxin. This rapidly increased in amount until in a short time the lymph contained one-third as much as the blood. This relationship between the blood and the lymph continued for several days, the antitoxin in both gradually lessening. The same experiment was tried with the tetanus toxin, and within 15 minutes the lymph was strongly toxic. This relationship continued, the amount in both blood and lymph gradually diminishing.

A final experiment was then made by injecting a dog with the tetanus toxin. After 24 hours the thoracic duct was tapped and the lymph tested. Each cubic centimeter was found to contain 45 fatal doses for a gram of mouse. A large injection of antitoxin for each gram of dog was then injected intravenously, and lymph specimens taken from time to time. The result of the test showed that during the first 15 minutes the lymph continued with undiminished toxicity. During the next 15 minutes toxicity dropped to one-half the amount, and in the next 15 minutes it became neutral. At the end of an hour the lymph was antitoxic. The results showed that an intravenous injection of antitoxin immediately neutralizes the blood, and in about 30 minutes, or shortly after, makes the lymph antitoxic. The spinal fluid is much slower than the lymph in showing antitoxin, and it never accumulates to any great extent, the final ratio being 1 to 100.

In 1898 Roux and Borrel suggested the treatment of tetanus through the direct injection of antitoxin into the central nervous system by cerebral or lumbar injection. They considered that they got better results
than from subcutaneous injections. Ransom investigated this matter and found that a subdural injection is practically the same as injecting anywhere in the subarachnoid space. He found that after subarachnoid injection, either in the region of the brain or the spinal cord, the antitoxin rapidly passes by way of the lymph into the blood, so that all but a trace has disappeared within 24 hours. He found that the tissues of the central nervous system contained no antitoxin, and that hardly a trace remained in the spinal fluid. He then injected tetanus toxin in the subarachnoid space, both by injecting through the brain tissue and by lumbar puncture. He found that a portion of the toxin appeared in the blood, while a large portion remained attached to the central nervous system, and that after such an injection the substance of the central nervous system lost its normal power to neutralize toxin and had become toxic. He proved that this was not because of any remaining toxin in the cerebrospinal fluid. He also found that the spinal cord matter always contained more toxin than that of the brain. He found that when moderate amounts were injected the blood contained no antitoxin, while the brain and spinal substance were toxic.

Absorption of Tetanus Antitoxin from the Subcutaneous Tissue of Man.—In order to test the absorption of tetanus antitoxin in man, and to learn the length of time it remained in the blood, I injected a healthy adult subcutaneously with 10,000 units of antitoxin. The results, as tested in bleedings taken at intervals during six days, were as follows:

<table>
<thead>
<tr>
<th>Time</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 hours</td>
<td>0.5</td>
</tr>
<tr>
<td>24 hours</td>
<td>0.8</td>
</tr>
<tr>
<td>48 hours</td>
<td>1.0</td>
</tr>
<tr>
<td>72 hours</td>
<td>1.0</td>
</tr>
<tr>
<td>144 hours</td>
<td>0.8</td>
</tr>
</tbody>
</table>

The charts of cases of diphtheria (see page 462) injected either subcutaneously or intravenously are of interest, as they undoubtedly parallel cases of tetanus injected with tetanus antitoxin.

Results of the Use of Antitoxin for Immunisation.—The striking results which have been obtained, both in human and in veterinary practice, with the prophylactic injection of tetanus antitoxin, would seem to warrant the treating of patients with immunizing doses of serum—at least in neighborhoods where tetanus is not uncommon—when the lacerated and dirty condition of their wounds may indicate the possibility of tetanus infection.

Splendid results have followed this practice in many places. It is the custom at many dispensaries in New York City and elsewhere to immunize all Fourth of July wounds by injecting 1,000 units. None of these have ever developed tetanus. Even the few cases of human tetanus reported as occurring after single injections of antitoxin prove the value
of immunizing injections, for the mortality was low. They teach, however, that where later tetanus infection is suspected the antitoxic serum should be given a second, and even a third, time at intervals of seven days.

With Dr. Matthias Nicoll, Jr., I have recently compared subcutaneous, intravenous, intraneural, and intraspinal injections. The results with intraspinal injections were considerably better than with intravenous and those with intravenous injections did much better than those receiving subcutaneous injections. The intraneural injections had no appreciable effect. The units required by the intraspinal method were less
### TABLE III

Comparison of Results of Treating Tetanus in Guinea-pigs by Intracardial, Intraneural and Intraspinal Injections of Antitoxin

March 24, eighteen pigs injected in hind leg with two minimal fatal doses of toxin. March 25, twelve of these were given antitoxin as shown 17½ to 18 hours later, inoculated leg being stiff in the degree noted at the time. The remaining six were bled until 22½ to 23 hours after inoculation, as the stiffness was not marked until that time.

<table>
<thead>
<tr>
<th>Number</th>
<th>Weight (Grams)</th>
<th>Condition of Leg</th>
<th>Method</th>
<th>Amount</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>116</td>
<td>290</td>
<td>fairly stiff</td>
<td>control</td>
<td>100 units</td>
<td>T 3 days</td>
</tr>
<tr>
<td>42</td>
<td>310</td>
<td>fairly stiff</td>
<td>control</td>
<td></td>
<td>T 3 days</td>
</tr>
<tr>
<td>296</td>
<td>250</td>
<td>slightly stiff</td>
<td>Heart</td>
<td>100 units</td>
<td>T 8 days</td>
</tr>
<tr>
<td>227</td>
<td>275</td>
<td>fairly stiff</td>
<td>H</td>
<td>100 units</td>
<td>T 4 days</td>
</tr>
<tr>
<td>399</td>
<td>300</td>
<td>fairly stiff</td>
<td>H</td>
<td>100 units</td>
<td>T 5 days</td>
</tr>
<tr>
<td>316</td>
<td>255</td>
<td>slightly stiff</td>
<td>N</td>
<td>200 units</td>
<td>T 4 days</td>
</tr>
<tr>
<td>287</td>
<td>255</td>
<td>fairly stiff</td>
<td>N</td>
<td>200 units</td>
<td>T 3 days</td>
</tr>
<tr>
<td>879</td>
<td>265</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>193</td>
<td>305</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>320</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>289</td>
<td>280</td>
<td>fairly stiff</td>
<td>Nerve</td>
<td>200 units</td>
<td>T 3 days</td>
</tr>
<tr>
<td>306</td>
<td>285</td>
<td>slightly stiff</td>
<td>N</td>
<td>200 units</td>
<td>T 3 days</td>
</tr>
<tr>
<td>59</td>
<td>255</td>
<td>stiff</td>
<td>Spinal Canal</td>
<td>10 units</td>
<td>Disch. normal 4/23</td>
</tr>
<tr>
<td>304</td>
<td>275</td>
<td>fairly stiff</td>
<td>Spinal Canal</td>
<td>10 units</td>
<td>Disch. well, 4/23</td>
</tr>
<tr>
<td>321</td>
<td>320</td>
<td>fairly stiff</td>
<td>Spinal Canal</td>
<td>10 units</td>
<td>Disch. well, 4/23</td>
</tr>
</tbody>
</table>

than by the other methods. Repeated large injections did not give any better results than a single sufficiently large injection. The above table gives the striking results obtained in one representative experiment.

**Results in Man.**—In actual cases in which the treatment was given within six hours of the development of symptoms the results observed by us have been surprisingly good. The recoveries in the cases treated by intraspinal injections have been over 70 per cent. In some cases no beneficial results appeared. We have seen numerous cases of generalized tetanus that, after a moderate intraspinal and large intravenous injection, have markedly improved, and finally recovered, and these cases have certainly done much better than apparently similar ones receiving palliative treatment alone. Lambert, who, some years ago, made an exhaustive study of tetanus, states that in a total of 114 cases of this disease treated with antitoxin by the older method, according to published and unpublished reports, there was a mortality of 40.35 per cent. Of these 47 were acute cases—that is, cases with an incubation period of eight days or less, and with rapid onset, or cases with a longer period of incubation, but intensely rapid onset of symptoms; of these the mortality was 74.46 per cent. Of the chronic type—those with an incubation period of nine days or more, or those with shorter incubation with slow onset—there were 61 cases with a mortality of 16.39 per cent. With a still larger number of cases the results indicate that with tetanus antitoxin about 20
per cent, better results are obtained than without. I have always believed that when antitoxin is given more promptly, in sufficient first doses and by the best methods, the results will be much better than those quoted by Lambert. The results recently tabulated by Ernest E. Irons bear out this opinion. All but twenty of the 245 cases were treated in large hospitals. He has kindly furnished me two of his tables for use in this article. (See pages 464 and 465.)

The cases tabulated by Dr. Irons apparently demonstrated that cases treated with antitoxin did better than those not receiving it, and those having large doses better than those receiving small doses. The examination of the last table would apparently show that those receiving the injections on the second and third days did better than those receiving them on the first. This is doubtlessly due to the fact that the most acute cases were those which came first to the attention of the surgeon or the physician and, therefore, received antitoxin on the first day. Those in which the tetanus developed slowly delayed seeking treatment and, therefore, one or two days elapsed. Such cases, if they had been acute would have been dead before the time they received their treatment. Even those receiving treat-

<table>
<thead>
<tr>
<th>TABLE IV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cases Treated with Serum</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Incubation (Days)</th>
<th>Total Cases</th>
<th>Died</th>
<th>Recovered</th>
<th>Mortality (Per Cent.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Five and less...</td>
<td>38</td>
<td>27</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Six...</td>
<td>18</td>
<td>15</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Seven...</td>
<td>21</td>
<td>16</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Eight...</td>
<td>17</td>
<td>14</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Nine...</td>
<td>24</td>
<td>17</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Ten...</td>
<td>13</td>
<td>7</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>131</td>
<td>96</td>
<td>35</td>
<td>73.28</td>
</tr>
<tr>
<td>Eleven to fifteen</td>
<td>47</td>
<td>22</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Sixteen and over</td>
<td>22</td>
<td>6</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>69</td>
<td>28</td>
<td>41</td>
<td>40.57</td>
</tr>
<tr>
<td>Total all cases incubation known</td>
<td>200</td>
<td>124</td>
<td>76</td>
<td>62.</td>
</tr>
<tr>
<td>Incubation unknown</td>
<td>25</td>
<td>15</td>
<td>10</td>
<td>60.</td>
</tr>
<tr>
<td>Total all cases receiving serum...</td>
<td>225</td>
<td>139</td>
<td>86</td>
<td>61.77</td>
</tr>
</tbody>
</table>

**Cases with No Serum**

<table>
<thead>
<tr>
<th>Incubation (Days)</th>
<th>Total Cases</th>
<th>Died</th>
<th>Recovered</th>
<th>Mortality (Per Cent.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ten or less...</td>
<td>11</td>
<td>10</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Over 10...</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Unknown...</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>17</td>
<td>3</td>
<td>85.</td>
</tr>
</tbody>
</table>
TABLE V
Results with Respect to (1) Time when Serum Was Given, (2) Size* of Dose in First 24 Hours

A. Cases receiving first serum within 24 hours of appearance of first symptoms.

<table>
<thead>
<tr>
<th>Incubation (Days)</th>
<th>Large Doses</th>
<th>Small Doses</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Died</td>
<td>Recovered</td>
<td>Died</td>
</tr>
<tr>
<td>Ten or less</td>
<td>41</td>
<td>13</td>
<td>21</td>
</tr>
<tr>
<td>Over ten</td>
<td>11</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td>Totals</td>
<td>52</td>
<td>28</td>
<td>27</td>
</tr>
</tbody>
</table>

B. Cases receiving first serum in second 24 hours after appearance of first symptoms.

<table>
<thead>
<tr>
<th>Incubation (Days)</th>
<th>Large Doses</th>
<th>Small Doses</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Died</td>
<td>Recovered</td>
<td>Died</td>
</tr>
<tr>
<td>10 or less</td>
<td>11</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Over ten</td>
<td>2</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Totals</td>
<td>13</td>
<td>17</td>
<td>7</td>
</tr>
</tbody>
</table>

C. Cases receiving first serum over 48 hours after first symptoms.

<table>
<thead>
<tr>
<th>Incubation (Days)</th>
<th>Large Doses</th>
<th>Small Doses</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Died</td>
<td>Recovered</td>
<td>Died</td>
</tr>
<tr>
<td>Ten or less</td>
<td>10</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Over ten</td>
<td>7</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Totals</td>
<td>17</td>
<td>16</td>
<td>8</td>
</tr>
</tbody>
</table>

D. Grand Totals

<table>
<thead>
<tr>
<th>Incubation (Days)</th>
<th>Large Doses</th>
<th>Small Doses</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Died</td>
<td>Recovered</td>
<td>Died</td>
</tr>
<tr>
<td>Ten or less</td>
<td>62</td>
<td>28</td>
<td>34</td>
</tr>
<tr>
<td>Over ten</td>
<td>20</td>
<td>33</td>
<td>8</td>
</tr>
<tr>
<td>Grand Totals</td>
<td>82</td>
<td>61</td>
<td>42</td>
</tr>
</tbody>
</table>

* A small dose 3000 units or less subcutaneous. A large dose over 3000 units subcutaneous or 3000 or less intraspinal or intravenous.
ment in the first twenty-four hours should, if that was in the later hours of the day, be considered as receiving injections late. There is no question that every hour counts and that those receiving intraspinal or intravenous injections within the first few hours of definite symptoms show a much greater percentage of recovery than those given in the table by Dr. Irons. During the past two years intraspinal injections have been given in nearly every case occurring in New York City. Dr. Nicoll and I collected the first twenty cases. The results showed 80 per cent. of recoveries.

In judging the effect of antitoxin given intraspinally in this series of cases, it must be remembered that the patients were not selected, but that every case of tetanus reported was given the benefit of the treatment regardless of the clinical condition. The series, therefore, may be said to be fairly representative of the type of the disease occurring in and about the city of New York. A few of these patients would undoubtedly have recovered if the intraspinal injection of antitoxin had not been given or, indeed, without any treatment other than symptomatic. The results obtained, however, in the saving of life are so much more favorable than those in previous years, when large doses of antitoxin were recommended to be given by the intravenous and subcutaneous methods, that there can be no reasonable doubt that the low death rate, 20 per cent., here obtained was largely due to intraspinal dosage.

**The Actual Antitoxic Treatment of a Case of Tetanus**

A case of tetanus should be injected at the first possible moment after the development of suspicious symptoms.

The best results are obtained through intraspinal injections, the next through intravenous. Subcutaneous injections are much less efficacious because of the slow absorption of the antitoxin. Injection into the ventricles of the brain is more dangerous than by the intraspinal way, and presents no advantages. An injection into the trunk of the nerve supplying the infected part is theoretically of value, but when an intraspinal, or even an intravenous, injection has been made it is of no practical value. Injection of antitoxin into the tissue of the cord itself is unnecessary, and does not add to the protection given by the intraspinal way. The intraspinal injection in an infant or child should be from 500 to 2,000 units, according to its size; in an adult 3,000 to 5,000 units.

The amount of fluid should be as large as can be injected, without causing pressure symptoms, so as to spread as thoroughly as possible throughout the subdural space. If the serum is thick it should be diluted with normal salt solution or sterile water.

The patient should lie on the right side with the knees drawn up and the left shoulder depressed. The skin of the patient's back, the hands of the operator, and the syringe should be sterile. The needle should be
SPECIFIC TREATMENT OF TETANUS

4 cm. in length, with a diameter of 1 mm. for children, longer for adults.

The puncture is generally made between the third and fourth lumbar vertebrae. The thumb of the left hand is pressed between the spinous processes, and the point of the needle is inserted in the median line, or a little to the right of it, on a level with the thumb nail, and directed slightly upward and inward toward the median line. At a depth of 3 or 4 cm. in children and 7 or 8 cm. in adults the needle enters the sub-arachnoid space, and on withdrawing the obturator the fluid flows out in drops or in a stream. After the flow of fluid has stopped a container holding the thinned antitoxic solution is connected by a short rubber tube to the needle, and the requisite amount of antitoxic fluid allowed to run in by gravity. If for any reason sufficient fluid will not enter the canal gentle pressure is used. Use 5 c. c. to 20 c. c., according to age.

The amount of antitoxin advised to be injected is many hundred times as much as is necessary to neutralize the toxin, if only it can reach it. The antitoxic fluid should be warmed to blood heat.

Besides the intraspinal injection, an intravenous injection should be given so as to immediately neutralize the toxin in the blood, and soon afterward that in the lymph. The size of the individual, rather than the severity of the case, determines the amount to be given, for, in tetanus, every case is very grave. A good rule is to give 2,000 units for every 10 pounds. A child of 40 pounds would receive 8,000 units. The serum should be warmed to body heat and given slowly. All precautions to avoid infection should be used, so far as the general body is concerned.

These two injections practically suffice for the antitoxin treatment, as the blood will remain strongly antitoxic for five days. This is plainly seen in Figure 2, showing the antitoxin in the blood after the lapse of a week. The intraspinal injections had better be repeated after 24 and 48 hours. The antitoxin rapidly passes from the spinal fluid to the blood and it is possible that some toxin may enter the cord from the nerve trunks. The repeated injections certainly do no harm and seem to do good. The important thing is to give enough at the first possible moment. On the fifth day a subcutaneous injection of 10,000 units is advisable in order to keep up the antitoxic strength of the blood for the next week, so that if toxin may still be developing in sloughing fissures it will be harmless.

When one is unable to give the antitoxin intraspinally or intravenously, then it should be given subcutaneously without delay, and if possible a later intraspinal injection can be given. This, however, is of far less value than the intraspinal injection. Actual histories of a few cases with both the antitoxic and general treatment are given in Vol. II, Chapter XXI. The amount should be twice as much as when given intravenously. When the amount of antitoxin available is less than the desired amount it should be given immediately, and then later, when a further supply is obtained, the remainder should be given.
CHAPTER XVIII

PNEUMOCOCCUS INFECTION

RUFUS I. COLE and A. R. DOCHEZ

GENERAL CONSIDERATIONS

Diplococcus pneumoniae (Weichselbaum, 72) or the pneumococcus (Fraenkel, 25), as it is commonly called, is a highly parasitic coccus which is widely distributed throughout nearly all habitable parts of the world. In the tropics and the regions where extreme cold prevails during a large portion of the year, the organism is much less frequently found than in the temperate zones where seasonal variations in temperature and climatic conditions are more extreme. Pneumococcus infections may, however, show a high degree of incidence in tropical and subtropical climates, affecting most severely the natives of these localities. Where such a condition has prevailed, it has followed the association of natives, among whom in their normal habitat pneumococcus infection was nearly unknown, with whites coming from regions where pneumonia was common and who probably acted as carriers of the infection. The high susceptibility of natives in such an epidemic indicates the probable absence of previous exposure to pneumococcus infection.

Although Eberth, Klebs and Koch described cocci resembling pneumococcus found in association with lobar pneumonia, the cultural methods at their disposal were insufficient for a positive identification of the organism. The discovery of the pneumococcus may be attributed to Sternberg (67) and to Pasteur (60), who published almost simultaneously accounts of the lance-shaped diplococcus in the normal mouth, which was able to induce a fatal septicemia in rabbits. They, however, did not associate the organism of the mouth with the various pathological lesions which we now know to be caused by pneumococcus, and it was only after the thorough studies of Fraenkel and of Weichselbaum that the constant association of the pneumococcus with lobar pneumonia was satisfactorily determined.

The pneumococcus is occasionally met with as an infectious agent in lower animals, but it is in man that the organism finds its most favorable habitat. It is known to occur, at least at times, as a harmless inhabitant of the buccal cavity in from fifty to seventy per cent. of normal indi-
individuals. As a pathological agent it is found in a variety of disease conditions among human beings. General invasion of the blood by the pneumococcus without evident local lesion has been reported. It would seem probable, however, that such a condition must be extremely rare and that in most of these cases some hidden focus has been overlooked. At least in one apparent case of this type, after diligent search a small alveolar abscess was found which served as the portal of entry. Focal lesions are by far the most common manifestations of pneumococcus infection in man. Of these, lobar pneumonia, with its complications and sequelae, is the most important. Pneumococcus may, however, produce the lobular type of pneumonia, and is a common concomitant infection in ordinary colds and disease of the accessory sinuses of the nose. It may occur as an independent agent in disease of the middle ear, ulcer of the cornea, in purulent meningitis, in acute arthritis, and in peritonitis. Many of the focal localizations of the organism outside of the lungs, however, represent metastatic infections derived from a primary site in the lung.

The chief importance of the pneumococcus lies in its ability to produce a croupous inflammation of the lungs, which is the severest and most fatal of the acute infections which are common to temperate climates. Acute lobar pneumonia, because of its striking and characteristic clinical picture, has been recognized since the earliest times. The recognition of the disease as a definite clinical and pathological entity is the result of the eminent studies of Morgagni, Baillie, Laennec, Rokitansky and Addison.

Lobar pneumonia is an endemic and generally sporadic disease that is common throughout the United States and Canada. It is frequent all over temperate Europe, in the inhabited portions of the south temperate zone, such as Australia, parts of South America, and in South Africa. Although it is much less frequent in the tropics, it is often seen even here among the inhabitants of the plateau regions. The census of 1900 showed that in the United States somewhat over ten per cent. of all deaths were due to some variety of pneumonia. Some statistics seem to indicate that the incidence of pneumonia is increasing. That this apparent increase may be due to better methods of diagnosis is very probable. However, one may safely say that the general incidence of pneumonia has shown no tendency to diminish. This may be due in part to the general acceptance of the view of the non-contagousness of pneumonia and the consequent lack of measures of prevention. During the same period of time such diseases as diphtheria and tuberculosis have shown a quite definite shrinkage, and one feels tempted to ascribe this to the widespread activity directed toward the limitation of these diseases. It seems to be true that the incidence and fatality of pneumonia may vary from year to year, but this is most probably associated with differences in climatic conditions. It is also possible that wave-like changes in the
virulence of pneumococcus races as a whole may occur, or that the incidence of infections with the more virulent races may be more common in one year than another. In view of the fact that most individuals harbor in the mouth an organism indistinguishable from the pneumococcus, the presumption is that most pneumonic infections are auto-infections, and that the important factor in determining the incidence of the disease is a variation in individual susceptibility. Dochez and Avery (16a) have recently shown that pneumococci belonging to what are known as Groups I and II do not occur in the mouth secretions of healthy persons unless such individuals have been in intimate contact with cases of pneumonia in which infection was due to these types of pneumococci. Such an observation indicates that infection with these varieties of pneumococcus spreads either through contact with an infected individual or through association with a healthy carrier. Definite epidemics of pneumonia are not of infrequent occurrence, and generally prevail where highly susceptible individuals are exposed to infection or among persons living in close association. Such epidemics have developed as a rule in schools, hospital wards, prisons and on ship-board. Unfortunately a bacteriological diagnosis of the type of pneumonia has been made in only isolated instances.

Owing to the previous lack of a well-defined epidemiology and the absence of sufficient evidence showing the dependence of one case of pneumonia upon association with some preceding case, we have been forced to conclude that exposure is universal and that the incidence of the disease is determined by special conditions in the individual. Certain factors have a more or less immediate influence upon the occurrence of the disease. Statistics teach that pneumonia is commonest in early adult life, the period of greatest physical activity, though the mortality is greatest among the aged. Those who labor out of doors are more often affected than those engaged in sedentary occupations. Both of these factors indicate that fatigue, especially when accompanied by exposure to unfavorable climatic conditions, has an important influence upon resistance. Previous irritation or infection of the respiratory passages seems to act as a predisposing factor in the causation of pneumonia. At least fifty per cent. of all patients give a history of a "cold" for variable periods preceding the acute onset. Whether such colds are of pneumococcus origin and the pneumonia simply represents an extension of the infection is not known at the present time. Alcohol depresses the general resistance, increases liability to exposure, and has an influence in the causation of certain cases. The predisposing effect of previous attacks is of doubtful significance as we know that various races of pneumococcus exist, and though infection with one race may confer a permanent immunity against that race, it may have no effect against infection with heterologous races.

Until study of the epidemiology becomes more widespread but little
hope exists that the disease can be attacked from the standpoint of prophylaxis, and we must look forward for a time, at least, to a continued high incidence and mortality that is appalling. The physician is, therefore, reduced to consider what effective measures exist for the successful handling of the individual who is suffering from an acute attack of the disease. The problem of directly influencing the normal course of pneumonia is extremely complex and attended by what appear to be almost insurmountable difficulties. The pathological process is a rapidly developing one and the clinical onset usually fulminant and without warning. Often when the physician first sees the patient, the lungs may already be the seat of widespread infection. Of favorable import, however, is the tendency of the disease to become localized in a single lobe, and in the majority of favorable cases for this localization to be rendered permanent by rising resistance of the infected individual. Once localization is successfully accomplished, the severity of the symptoms seems to abate somewhat. The margin of safety is, nevertheless, a narrow one, and, if the virulence of the infecting organism is great or the resistance of the patient unduly low, a spread of the infectious process almost always occurs. With a spread of the process after the initial involvement, the symptoms again become increasingly severe, and it is then that the struggle for life reaches a most precarious stage, for it is during the period of such an active growth of the pneumococcus that the already weakened patient is most likely to succumb. A progression of the disease may manifest itself in two ways. There may be an increase in the area of lung involvement, and with each successive lobe that becomes diseased the picture grows more hopeless. On the other hand, the lesion in the lung may appear to be stationary and, in spite of this, the patient rapidly loses ground and dies on from the fifth to the seventh day of the disease. Usually in such cases a serious invasion of the blood has occurred, and the pneumococcus, finding a favorable medium for its growth, develops rapidly and death is due to an overwhelming septicemia. Bacterial counts of the organisms in the blood in these cases have been found to range from one to sixty-five thousand per cubic centimeter. Often both processes occur at the same time and with the active spread of consolidation of the lung, there is a simultaneous growth of the pneumococcus in the blood. If an efficient specific therapy is to be developed, it must meet the gravity of the situation in such severe cases and must be able to match the extraordinary rapidity with which these phenomena of the disease arise.

When confronted with an established bacterial infection, the physician has at his disposal but a very limited number of methods by means of which he can hope to influence the course of the process favorably. In the majority of instances his attempts must represent an effort to aid the lines of defense already provided by nature, or, at most, to relieve the patient of controllable embarrassments. In a few instances the medical
sciences have provided us with agents which either attack directly the
invading micro-organism or neutralize the products, by means of which
they intoxicate and destroy the host. The latter methods offer the most
hopeful means of controlling an established bacterial infection, and it is
to the search for such specific methods of therapy that much of the inves-
tigation of infectious diseases is at the present time directed. Until re-
cently the artificial production of specific therapeutic agents has been
carried on entirely in the bodies of foreign animals, or else efforts have
been made to provoke by special methods, such as vaccination, an in-
creased production of specific antibodies within the body of the host him-
self. Recently the introduction by Ehrlich into the therapy of disease
of a synthetic chemical compound with specific anti-bacterial action has
greatly enlarged the field of specific therapeutics. All of the methods men-
tioned here have been tried from time to time in the treatment of lobar
pneumonia.

Pneumonia belongs to a group of diseases which may be styled self-
limited. Practically nothing can be done by ordinary methods to shorten
the course of the disease, and recovery, when it occurs, is usually sharp
and spontaneous. The rapidity with which the patient passes from a
condition of extreme gravity to one of comparative safety suggests the
occurrence of some quite sharp and definite reaction against the infecting
parasite on the part of the host. Studies of the blood of individuals
recovering from infective diseases have shown that at some stage of the
process in many cases certain agents known as antibodies develop, which
may exhibit a variety of specific effects upon the micro-organism causing
the disease. They may belong to the groups of agglutinins, bacteriolysins,
opsonins, protective bodies of unknown action, or other bodies with specific
reactions. The artificial production of such bodies in animals by injec-
tion of dead or living pneumococci has been comparatively easy. F. and
G. Klemperer (35) during the early years of the study of immunity
demonstrated that rabbits injected with the pneumococcus or its products
in culture developed in their blood serum a power to prevent infection
of normal rabbits with large doses of living virulent pneumococci. The
demonstration of the presence of such bodies in the blood of patients re-
covering from pneumonia and the relation of the appearance of these
bodies to the crisis has been somewhat more difficult, and their presence
and influence were doubted for many years. Recent studies have, how-
ever, shown that in most instances protective antibodies occur during the
course of lobar pneumonia, and the conclusion seems justified that they
play at least some rôle in the mechanism of recovery. The confirmation
of these results has been of great importance, because, without such a
basis for investigation, little hope could be entertained of making prog-
ress in the artificial production of such bodies and their use as therapeu-
tic agents. There seems, then, to be sufficient scientific background to
encourage the serious consideration of the usefulness of biological bodies which may be supplied artificially from the bodies of foreign animals or produced by special methods in the body of the host himself.

Consideration must also be given to the possible efficiency of some of the synthetic drugs which have recently been developed and for which a specific action is claimed. These drugs have been used independently and in some cases in conjunction with specific antisera. Products of animal cells have been utilized in the treatment of pneumonia, and certain chemical substances which acted, not against the infectious agent, but which provoked some special type of cellular reaction on the part of the host. All these various measures can probably be brought together and considered under the heading of specific therapy. Undoubtedly the most important are those which have in view the development of specific biological agents, such as serotherapy and vaccination, or the production of chemical bodies with specific antibacterial action.

**SERUM THERAPY**

Attempts to control bacterial infections by means of specific antisera depend upon either one of two types of action which these sera possess. Their activity may be directed either against the living organism itself and result in its death or a limitation of its ability to develop, or it may be directed against products of the bacterial cells which are diffusible and which may be able to effect injury at a distance where no living bacterial cells are present. The first type of sera are known as antibacterial or anti-infectious, the second as antitoxic. Antitoxic sera, such as we have in the case of diphtheria and tetanus, have proved the most efficacious of the antisera which have been produced so far. Attention of investigators was early directed to the search for toxins produced by the pneumococcus and to attempts to develop an immunity to such possible bodies. So far the demonstration of a soluble toxin derived from the bacterial cells of pneumococcus that is in any way comparable to diphtheria toxin has not been successful. The Klemperers (35) tested the toxicity of broth cultures from which the bacteria had been removed. Although it was possible to kill animals with this material, such large quantities were required as to render it unlikely that the toxic action could be due to the presence of substances analogous to true toxins. These solutions also possessed some immunizing qualities which were dependent, doubtless, upon the presence of a certain quantity of bacterial protein derived from disintegrated organisms. That the pneumococcus does not, under the ordinary circumstances of bacterial growth, form highly toxic bodies, and that even large doses of the living bacterial bodies can be given without toxic action unassociated with a general bacterial infec-
PNEUMOCOCCUS INFECTION

tion, render it unlikely that an antitoxic serum of the type of diphtheria antitoxin can be produced. More recently substances have been prepared from bacterial bodies by special methods which seem to be more nearly related to the soluble toxins. These substances produce the type of death seen in acute anaphylactic shock, and have been tested largely on such susceptible animals as the guinea-pig. Friedberger (7), who was the first to prepare these bodies from bacteria, has called them anaphylatoxins, and is inclined to attribute the intoxication arising in infectious diseases to substances of this nature. Jold (17) first prepared such a substance from the pneumococcus. By submitting pneumococcus to the action of a specific antibacterial serum and subsequently digesting the sensitized bacterial bodies with guinea-pig complement, a toxic body is formed which kills guinea-pigs acutely in a few minutes. The mode of death resembles very much that seen in acute anaphylactic shock. Substances of like nature have been subsequently prepared by Rosenow (64) by allowing the bodies of the pneumococcus to undergo autolysis in salt solution, and by Cole (13) by dissolving the bacteria in bile, in which they are readily soluble. Attempts to immunize animals against these bodies so far have been failures, although antibacterial sera prepared from horses by the injection of living virulent pneumococci may have a slight neutralizing effect. General opinion holds that these substances are not toxins of the type of diphtheria toxin, which is probably a true protein, but represent some intermediate stage in the digestion of bacterial protein which is toxic. Support is lent to this view by the fact that when bacterial digestion with complement or bacterial autolysis is allowed to go on for too long a time, the toxic qualities of the mixture disappear. On the other hand, the work of Cole suggests that these bodies may be preformed in the bacterial body and represent the endotoxins of Pfeiffer. It is by no means established as yet that the toxemia of infectious diseases is dependent upon such artificially produced bodies, and the fact that in all likelihood they are disintegration products of protein renders it unlikely that anything in the nature of antitoxic immunity can be developed against them.

Attempts to prepare specific antibacterial sera whose object is the destruction of the bacterial body have been more hopeful. Such sera are highly specific in their action, and for their proper preparation and use require a refined and detailed knowledge of the bacteriology of the infection in which they are to be used. Though pneumonia of a lobar type may be produced by organisms other than the pneumococcus, and, in some instances, such organisms may act in conjunction with the pneumococcus, for practical purposes in a study of the specific therapy of pneumonia it is sufficient to consider the pneumococcus alone as the causative agent.

Shortly after the definite establishment of the causal relationship of
the pneumococcus to lobar pneumonia by Fraenkel and by Weichselbaum, experimenters began to study the immunity-producing qualities of this organism. Attempts were first made to develop an active immunity in experimental animals. A. Fraenkel (26) made the fundamental observation that rabbits which had survived a subcutaneous injection of living pneumococcus were later immune against a subsequent injection of a fully virulent culture. Other observers later confirmed this result and were able to call forth an active immunity against the pneumococcus in a variety of ways. Foa and Bordoni-Ufreduzzi (22) were able to protect animals against fatal doses of virulent pneumococci by previously injecting them with attenuated cultures of pneumococcus. G. and F. Klemperer (35) obtained active immunity by the use of cultures killed either by heat or by the addition of carbolic acid. Emmerich (18) and also Mennes (46) were able to get a high degree of active immunity by first treating their animals with killed or attenuated cultures and later submitting them to injection with increasing doses of living, highly virulent organisms. The later work of Neufeld (51) indicates that the highest degree of active immunity can be obtained in this way. Other means and various products of the pneumococcus have been used for active immunization, but the evidence favors the use of living virulent bacteria as the most useful method.

As soon as it had been determined that animals could be actively immunized against pneumococcus, observers turned their attention to the practical use that might be made of this phenomenon in the treatment of lobar pneumonia in man. Efforts were first made to transfer the immune principles developed in an actively immunized animal to other animals, which were then exposed to experimental infection. These experiments were early successful and a number of investigators have been able to protect animals against experimental infection with pneumococcus by giving either previously or simultaneously with the infecting dose a small quantity of the blood serum of an actively immunized animal. The results of treatment in animals, however, as contrasted with prevention or protection, have not been so satisfactory. While a very small amount of serum will usually protect an animal from a large dose of bacteria given with the serum or a very short time afterward, even a large amount of serum usually will not cure the animal after infection is well advanced. Evidence is not lacking, however, that even in animals such immune serums may have curative as well as protective action. Efforts at treatment have usually been attempted in rabbits or mice, which are extremely susceptible to pneumococcus infection and in which the infection runs a very rapid course. When injections of pneumococci are made directly into the lungs of guinea-pigs, the infection runs a slower course and Neufeld and Ungerma nn (53) have shown that in such cases, if injections of even small amounts of serum are made as late as three hours
following the infection, recovery occurs in a large proportion of the animals. These experiments in the production of active and passive immunity in animals to pneumococcus are so striking and fundamental that it is little wonder that efforts to find methods for using the sera obtained therapeutically in man were begun more than twenty years ago by the Klemperer brothers, and are still being persisted in in a number of places where medical investigation is carried on.

Attempts to utilize the serum produced by immunization of animals as a curative agent in cases of human lobar pneumonia were first carried on by G. and F. Klemperer (36). They treated 18 human cases with serum derived from highly immunized rabbits. In some of these cases they observed a permanent fall in the temperature and in others only a temporary lowering. Their trials were not carried further, nor were those of Foà and Scabia (23) nor of Jansson (33), who also thought that they had obtained beneficial results by the use of immune rabbit serum.

Many attempts at treatment have been made with the use of sera obtained by immunization of the horse or the ass. Washbourne (71) reports the treatment of six cases with horse serum. Three of these seemed to be benefited, one died, and in the other no effects were noted. Pane (56), who has prepared an antipneumococcus serum by the immunization of the donkey, treated 32 human cases with this serum. All but 3 of those who were treated in the advanced stages of the disease recovered. According to Pane, the serum effects an improvement in the subjective condition and a lowering of the temperature. A number of other observers have used Pane's serum and report favorable results following its use. On the other hand, Banti and Pieraccini (5), who treated 21 cases with Pane's serum, failed to get any beneficial results. Spolverini (66), using the same serum in 11 cases, thought that the results were slightly favorable, but claims to have obtained the same effects by the use of normal horse serum. Eyre and Washbourne (20) have shown that samples of Pane's serum sent to them protected animals against infection with four strains of pneumococcus which they had, but failed completely to protect against a fifth strain. Cantieri (11) found that Pane's serum influenced somewhat the fever and general condition of the cases he treated, but had no noticeable effect on the outcome of the disease. In America Anders (3) has collected 535 cases of pneumonia which have been treated by specific serum. Of these, 474 received antipneumococcus serum, and 61 cases antidiphtheritic serum. Of these, 85 died showing a mortality of 18.3 per cent. Of course, those treated with antidiphtheritic serum should be excluded from the statistics, inasmuch as this could not be considered a form of specific serum therapy in pneumonia. Anders holds that the results observed in the serum-treated cases of pneumonia reviewed by him were not sufficiently favorable to warrant its introduction as a general method for the treatment of the disease. The majority of American
investigators who have employed antipneumococcus serum of the usual
type therapeutically, coincide with this view.

Certain observers, on account of the earlier doubtful results obtained,
have endeavored to interpret them and to improve the methods for the
production and administration of antipneumococcus serum. Tizzoni and
Panichi (70) have attributed the unfavorable results obtained from the
use of antipneumococcus serum to the fact that the organisms used for
the immunization of animals were grown on an unsuitable medium. To
correct this, they employed a specially prepared bouillon in which they
claimed that the pneumococcus formed toxins of the same character as
those formed in the animal body. They claim to have been able to kill
acutely animals injected with doses of such cultures. Animals were im-
munized first by the injection of filtrates and later by the full culture.
Care was taken in the time after injection of bleeding the animals, inas-
much as Tizzoni and Panichi found that the time of maximum concen-
tration of antibodies in the blood varied in different animals, and that
the high mark was of short duration. The authors obtained in this way
sera which in doses of 0.25 per cent. of the body weight of rabbits pro-
tected against a simultaneous intravenous injection of 0.2 c.c. of a virulent
pneumococcus culture, whereas the control animal died in twenty-four
hours. They were able also with like doses of serum and culture, the cul-
ture being given first subcutaneously, to cure rabbits after the appear-
ance of the pneumococcus in the blood. In one instance where larger
doses of serum were employed, an animal recovered when the control had
died before the test animal received the first dose of serum. Such results,
if reliable, indicate a serum of extraordinarily high potency. Panichi (57)
treated 7 cases of pneumonia with intravenous doses of from 15 c.c. to
30 c.c. of this serum and says that in all cases the administration of the
serum was followed by beneficial results, and a fall of the temperature by
lysis. In view of such striking experimental and therapeutic results, it is
surprising that no further observations on the action of the serum seem to
have been made.

Römer (62) sought to increase the efficiency of the serum prepared
by him in a different way. Instead of immunizing a single animal and
using the serum thus obtained, several animals were chosen, including
horses, cattle and sheep. After each had been immunized to a sufficient
degree, they were bled, the serum obtained mixed together and used for
treatment. By using antibodies derived from different sources, it was
hoped to obviate the possibility that certain individuals might fail to
complement the antibodies of the serum if these were derived from a sin-
gle source, whereas by furnishing a multiplicity of antibodies, the chances
of the treated individual’s possessing suitable complementing bodies were
increased. In the later methods of preparing the serum, this complicated
method was abandoned, as was also the use for purposes of animal immuni-
zation of strains of pneumococcus cultivated directly from human material. Single animals were used and these were immunized by the injection of multiple strains of living highly virulent organisms, a method previously recommended by Emmerich, Mennes and others. Römer's polyvalent serum, prepared both by the earlier and later methods, has been and is still used extensively, both in the treatment of ulcus serpens and in lobar pneumonia. A number of men have reported the character of the results obtained by the use of this serum. Pässler (59) treated 24 cases, of which 4 died and 20 recovered. In favorable cases the course of the disease was shortened. As a rule, in from 6 to 12 hours after the administration of the serum, a notable drop in temperature occurred. The infection seemed to assume a lighter character after the serum, the subjective feelings of the patient were improved, and the circulation was favorably influenced. In 6 cases crisis occurred after the first injection, and in 4 cases, after the second injection. The serum was administered in from 10 to 30 c.c. given subcutaneously. Crux (15) also obtained favorable results in 12 cases, observing a fall in temperature, beneficial influence on the pulse, and shortening of the course of the disease. Crux administered the serum in doses of from 2.5 to 5 c.c. subcutaneously, repeated in 24 hours. The quantities of serum given by this observer were so small that it seems doubtful if the effects observed could reasonably be attributed to the action of the serum. Knauth (27) treated 7 cases, all of whom recovered. He employed larger doses of serum, from 20 to 60 c.c. Beyer (7) observed some decrease in the mortality in 21 cases treated with Römer's serum. Other investigators did not obtain such favorable results. May (45) observed a favorable subjective effect, but no influence in hastening the crisis or on the course, temperature or extension to other lobes. Lindenstein (42) observed a favorable subjective effect and a drop in temperature following injection which, however, soon rose again to the previous height. Of 16 cases treated by Winkelmann (76) with doses of from 10 to 40 c.c., 5 died, showing a mortality of about 30 per cent. Steyrer (68) using large doses of serum could not produce a critical drop in the temperature. Jürgens (34) observed no favorable effects following the use of the serum. The combined 44 cases treated by Pässler, Winkelmann and Lindenstein showed a joint death-rate of 25 per cent., a result which is conclusive evidence against any marked influence on the mortality rate.

The studies of Neufeld and Händel (52) and their associates on the preparation and action of antipneumococcus serum seem to mark a very distinct advance over the methods employed by previous observers. In immunizing the horses from which the serum was obtained they employed large doses of living virulent pneumococci. The cultures selected depended upon a careful serological study of several strains of pneumococcus obtained from human material. Previous observers had recognized the
probability of the existence of different varieties of pneumococcus and in their immunization work frequently used a multiplicity of strains. The relation of one strain to another had, however, never been satisfactorily tested. The investigations of Neufeld and Händel were carried on with strains of pneumococcus isolated from cases of pneumonia. Sera of high potency were obtained from rabbits, donkeys and horses by immunization of these animals with a single strain of pneumococcus. The sera thus obtained protected to the same degree as with the original strain against most of the other highly virulent strains of pneumococcus in their possession. There were, however, certain strains which, although they could not be distinguished by ordinary methods from the strain of pneumococcus used for immunization, were not influenced in any degree by the action of the serum. Equally efficient immune sera could, however, be prepared from these strains, and it was furthermore found that these sera protected animals neither against the first type strain nor was there cross protection between these two atypical strains, as Neufeld calls them. These observations at once make it evident that the type of organism concerned in the production of any case of pneumonia is of primary importance from the standpoint of specific therapy. For the successful immunization of animals, strains must be employed which include as far as possible such types as are met with in cases of human infection. Failure to obtain good results in particular instances of the disease require an investigation of the type of organism concerned in such a case before it can be determined that the lack of success is due to failure of the serum and not to an attempt to influence a strain which is insusceptible to the action of the serum.

Neufeld and Händel (53) also contributed important observations on methods of titration of the potency of antipneumococcus serum, and on the dosage and best methods of administration. Previous investigators had paid little attention to the potency of their sera, whereas Neufeld and Händel developed a method for testing the protective value on animals. Mice were injected with a constant quantity of immune serum and shortly afterward with varying doses of a culture of pneumococcus of standard virulence. By such a method the virulence of the organism was determined and the number of fatal doses against which a given quantity of serum would protect. In this way it is possible to maintain some standard of efficiency of the serum.

In the earlier studies of the action of antipneumococcus serum in human cases relatively small doses administered subcutaneously were employed. Neufeld and Händel (53) have recommended the use of much larger doses intravenously. In titrating immune serum against varying doses of pneumococci by injection into mice, they have shown that a certain amount of serum in relation to body weight is required to protect. This amount protects against many times the lethal dose. On the other
hand, a slightly smaller dose may not protect at all, even against only a very small multiple of the minimal lethal dose. In other words, such a serum does not obey the law of multiple proportions, and to be efficacious, even against a very mild infection, it must be present in the body in a given concentration. This concentration they have called the "Schwellenwert" or threshold concentration. Reckoning from their experiments on mice, they estimate that in man the curative dose of the variety of serum tested by them must be at least 75 c.c. It is evident, therefore, that one reason why antipneumococcus serum has not been more efficacious in the past is that it has not been administered in sufficiently large doses.

The serum of Neufeld and Händel has recently been prepared commercially and a number of observers have reported the results obtained from its use. Weitz (73) treated 38 cases with apparently beneficial results. The initial dose of serum was from 10 to 40 c.c. This was repeated in 12 hours, and many of the cases received several injections. Of 16 cases treated on the second day, 12 showed an apparently abortive course. Among these was one individual who showed 900 colonies of pneumococci in 10 c.c. of blood taken before the first injection. Two cases were fever-free on the third day, 10 on the fourth day and one on the fifth day. In 3 there was no shortening of the course of the fever. One of these, an alcoholic, died. After death the blood and organs gave sterile cultures, although before the use of the serum 10 c.c. of blood gave from 2,000 to 3,000 colonies of pneumococcus. The day following the injection the same quantity of blood showed 21 colonies and the succeeding cultures were sterile. Of 9 cases treated on the third and fourth days of disease, 9 showed a normal temperature after two days of treatment. In 2 of these cases there was no noticeable effect on the temperature. Three of the patients died, but in these the infection was a mixed one, so that the result was not clear-cut. Of 7 cases treated first on the fifth and sixth day, 4 died. Weitz concludes that the serum of Neufeld and Händel exhibits a specific action in cases of lobar pneumonia, and that this action is most manifest when the patients are treated in the early stages of the disease. The report of Weitz is of especial interest in showing the effect of the serum upon general pneumococcus infection. In his experience no case had recovered which showed such large numbers of organisms in the blood as the two mentioned. Unfortunately, in this series of cases no attempt was made to determine whether the type of organism in each individual case was susceptible to the protective action of the serum employed.

A smaller number of cases treated with the Neufeld-Händel serum are reported by Geronne (29). In all 12 cases were treated, among them 3 children. In the earlier cases in which small doses of serum were used, 10 to 20 c.c., the results were not especially favorable. In the later cases Geronne increased the dose of serum to 40 to 80 c.c. and found that
in these cases there was a marked improvement in the general condition and lowering of the temperature and, in some instances, a shortening of the course of the disease. Normal sheep serum used in a certain number of control cases showed no such favorable results. Geronne observed that the course of the local condition in the lung was not noticeably affected by the use of immune serum. Neufeld points out that, according to the work of Rosenow (63), consolidation persists in the lung even after the disappearance of living pneumococci, and argues from this that the serum could not be expected to have much effect on the local condition once the disease is well established. He emphasizes, however, the importance of the general infection and thinks that in many cases of pneumonia this is the most serious element of the disease. In addition, he thinks that the serum has some influence in preventing the development of new areas of consolidation in other portions of the lung.

The authors of the present paper have been interested in pneumococcus infections, particularly lobar pneumonia, for the past six years. The work was taken up with the object of developing, if possible, some form of specific therapy. In order to obtain proper material for the immunization of horses, a large number of pneumococcus strains freshly obtained from cases of lobar pneumonia were studied by Dochez and Gillespie (16). These studies indicate certain important reasons why antipneumococcus serum may not have proved of value in the past, and explain why even the administration of very large doses early in the disease may prove of value in only a small proportion of cases. In the past antipneumococcus serum has been administered indiscriminately in all cases of pneumonia, no effort being made in the individual case to determine the nature of the bacterium causing the infection. It has long been known that characteristic lobar pneumonia may be caused by a number of other organisms besides the pneumococcus, such as streptococcus and influenza bacillus. It is well recognized that an antipneumococcus serum cannot be effective in case the disease is due to an organism other than the pneumococcus, since such serums are as rigidly specific in their immune reactions as is antitoxin for diphtheria toxin. It must be granted, however, that a large majority of the cases of typical lobar pneumonia are due to pneumococcus, so that if such a serum were efficacious against all such cases, the results of its administration would be manifest. Neufeld, as has been previously mentioned, found that an antipneumococcus serum prepared by him by the immunization of a horse with a given race of pneumococci was effective against the race of pneumococci used for immunization, and also against certain other races obtained from cases of pneumonia; but against still other races of typical pneumococci he found that it had practically no effect.

Dochez and Gillespie (15) have shown that pneumococci isolated from cases of pneumonia may be divided into four groups. The organ-
isms belonging to each of the first two groups are specific, as far as their immune reactions are concerned. An immune serum produced by the injection of a horse with a race belonging to Group I has a specific action against all the members of Group I, but has no effect on the organisms of any of the other groups. In like manner, an immune serum produced by the injection of a horse with a pneumococcus belonging to Group II is protective against all other members of this group, but has no effect against the members of any other group. In Group III are included the organism of the type of pneumococcus mucosus. So far it has not been possible to produce an active immune serum against organisms of this type. An immune serum produced from a member of any of the other groups has no protective power for any race belonging to this group. In Group IV are included all races against which Serums I and II are not effective, and which, from their cultural and pathogenic qualities, do not belong in Group III. Animals may readily be immunized against any member of this group, and the serum of the immunized animal is protective against the race used for immunization. In no instance, however, has this serum been found to be effective against any other variety belonging to this group, nor against any of the members of Groups I and II. This classification of the large number of strains studied has been made by testing out the protective value of the different types of sera prepared for white mice. By making use of specific agglutination, the same classification is arrived at as by the protection experiments.

It has become evident, therefore, that while a large majority of cases of pneumonia are due to pneumococcus, so far as immune reactions are concerned, the cases of pneumococcus pneumonia are caused by organisms of at least four different types, and from the point of view of specific therapy, this is equivalent to saying that they are due to at least four different organisms. In 300 cases of pneumonia studied, the number of cases found to be due to organisms of the four different groups is shown in the following table:

**TABLE I.**

<table>
<thead>
<tr>
<th>Type of Organism</th>
<th>Number of Cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>102</td>
<td>34</td>
</tr>
<tr>
<td>2</td>
<td>99</td>
<td>33</td>
</tr>
<tr>
<td>3 (Mucosus)</td>
<td>36</td>
<td>12</td>
</tr>
<tr>
<td>4 (Heterogeneous)</td>
<td>63</td>
<td>21</td>
</tr>
</tbody>
</table>

It is evident from these results that in studying the effects of an immune serum on patients with pneumonia, but slight conclusions can be
drawn from its indiscriminate employment in all cases. First we must know the type of organism used for its production, and second, it must be employed only in cases due to organisms of the type used in its preparation. So far it has been possible to produce a serum of high protective power against organisms of Type I, and a second serum, somewhat less efficacious, against organisms of Type II. The immune serum produced against organisms of Type III is of somewhat lower potency than that against Type II, and for this reason it has not seemed likely that it is of sufficient value for therapeutic use in man. It is manifestly impossible to utilize a specific serum in infections due to Type IV, inasmuch as each member of this group from a serological standpoint represents a distinct variety. So far as the treatment of pneumonia is concerned, this latter fact is of minor significance, since our observations indicate that pneumonia due to organisms of Type IV is of relatively slight severity, and most of the patients recover without specific treatment. The relative virulence for human beings of the different groups is shown in Table II.

TABLE II

<table>
<thead>
<tr>
<th>Cases Due to</th>
<th>Number of Patients</th>
<th>Died</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>30</td>
<td>10</td>
<td>33.33</td>
</tr>
<tr>
<td>Type II</td>
<td>30</td>
<td>10</td>
<td>33.33</td>
</tr>
<tr>
<td>Type III</td>
<td>15</td>
<td>7</td>
<td>46.66</td>
</tr>
<tr>
<td>Type IV</td>
<td>25</td>
<td>3</td>
<td>12.00</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>30</td>
<td>30.00</td>
</tr>
</tbody>
</table>

At present, therefore, the problem of serum therapy in pneumonia has resolved itself into treating the cases due to organisms of Type I and Type II with Serum I and Serum II. In order to treat the individual case, however, it is necessary to have a method of determining very promptly after the patient comes under observation the type of organism concerned. It has been found possible to do this by using the following method: When a patient with pneumonia comes under observation, a culture is immediately made from the blood and also one from a portion of sputum coughed up from the lung; or, when this is not possible, a culture is made directly from the lung by the insertion of a needle. This procedure seems to be without danger. When sputum can be obtained, a culture may be most rapidly obtained by injecting a portion of the sputum into the abdominal cavity of a mouse. After sufficient growth has occurred, usually in about six hours, the mouse is killed, the abdominal cavity washed out and the cells and fibrin thrown out by slow centrifugalization; a suspension of organisms is thus obtained. How-
ever the culture is obtained, the agglutination test is at once applied. If the organism fails to agglutinate with either Serum I or Serum II, it is, of course, useless to undertake serum treatment. If, however, one of the sera agglutinates the organism, treatment may be commenced at once with the appropriate serum.

In view of the facts described here, it is obvious that only the most irregular results could be expected from the employment of sera prepared from organisms not previously studied in regard to their group relationship, and administered in cases in which nothing was known concerning the type of infecting organisms. If these requisites are fulfilled, theoretically, at least, antipneumococcus serum might be rendered effective. Serum prepared and tested for specificity in this manner has now been used by the authors in a considerable number of cases of pneumonia. Treatment has been limited to injections with antipneumococcus serum Types I and II. Treatment of pneumonia with serum Type I has given very good results. In sixty-five cases so treated the mortality has been 7.5 per cent., which represents a considerable reduction in the mortality observed in untreated instances of infection with this type of organism. These statistics include one individual who was moribund at the time of the first treatment, and another who died from pulmonary embolism after recovery from the pneumonia. The use of serum Type II seems to be much less effective as a therapeutic measure. The reduction in mortality of treated cases of this type as compared with the untreated has been so small that we have discontinued the use of the whole serum and attempts are now being made by Chickering to increase the potency of this serum by the use of special methods of concentration.

The method of administration of the serum is as follows: On admission, 0.5 c. c. of serum is injected subcutaneously to discover if the patient is hypersensitive. As soon as the type of organism is determined, from 50 to 100 c. c. of serum, diluted one-half with salt solution, is injected intravenously. The condition of the patient serves as a guide in the later treatment. Usually the serum is not given oftener than every twelve hours. The patients treated received totals of from 100 to 700 c. c. of serum. The early determination of the type of organism is of great importance, since the earlier in the disease that serum treatment is inaugurated the greater are the chances of a favorable result.

In the absence of a large number of treated cases, the efficacy of serum therapy must be based on other criteria. The effect of this serum on the temperature has been as follows: After most of the injections a reaction occurs, the temperature usually rises and then falls, but does not necessarily remain low. In some instances the rise of temperature has been marked, in others the rise of temperature following an injection has been only a degree or so. In all the cases except the fatal ones, the serum apparently had an ultimate favorable effect in lowering the tem-
temperature and shortening the course of the disease, though, of course, it is difficult to be sure of this. In no instance was one injection of serum sufficient to bring on a crisis. All the patients seemed to feel better following the injection of the serum, and in a number of cases the apparent lessening in the degree of intoxication was very manifest.

In favorable cases when the treatment was commenced early, no extension of the involvement of the lung occurred. On the other hand, however, there was no especial tendency in the treated cases for the lung lesion to resolve rapidly. If anything, there seemed to be a tendency for resolution to be delayed in these cases. This has been noted by other observers in cases of pneumonia treated with immune serum. A number of patients treated with Type I serum have developed empyema following their pneumonia. All of these have recovered after the usual surgical procedures with the exception of one individual who died from a secondary streptococcus septicemia. In a small number of instances fluid has developed in the chests of serum-treated patients which on removal has been found to be relatively clear and on culture to be free from microorganisms.

More important than the foregoing criteria, however, are the following observations, since they have depended solely on objective procedures. First to be mentioned is the effect of the serum on the organisms in the blood. In 8 cases pneumococci were isolated from the blood before the treatment was commenced. In all cases the occurrence of pneumococcus in the blood has been carefully studied. Whenever a bacteremia has existed, the organisms with but one or two exceptions have disappeared from the blood after a single injection of serum, that is to say within an interval of from eight to twelve hours. In general, therefore, one large dose of serum seems sufficient to sterilize the blood, and the conclusion seems justifiable that if organisms are not present in the blood, the administration of serum will prevent their entrance.

In previous studies of the blood of patients with lobar pneumonia, it has been shown that, as a rule, the appearance of protective substances in the blood, when demonstrable, coincides rather sharply with the period of critical fall in temperature and the disappearance of symptoms. Before the crisis they are not present in the blood in any measurable degree. A similar study has been made of the protective substances in the serum in a number of cases of pneumonia treated with the authors’ immune serum. In all the cases studied it has been possible to demonstrate the appearance of such substances in considerable amounts in the serum following the administration of immune serum, even when this serum has been administered early in the disease, at a period when such protective substances are otherwise never present. These substances persist, and in case they play a part in the mechanism of recovery, as has been
concluded from previous studies, it is evident that their appearance indicates a favorable action of the immune serum.

The clinical and laboratory study of a series of cases of pneumonia treated by the injection of large amounts of appropriate serum seem to indicate that a method has been devised for the successful specific treatment of at least a portion of the cases of acute lobar pneumonia.

In reviewing the work done on the serum therapy of lobar pneumonia, one sees a continuous progress in the efficiency of the methods of production and administration of antipneumococcus serum. In the earlier observations but little attention was paid to the potency of the serum or to the characteristics of the organisms employed in its production. When it was used in human cases, the doses administered and the method of application were probably entirely inadequate in the majority of instances. The work of Neufeld and Händel emphasized the importance of paying attention to the strains of pneumococci used for the immunization of animals, and the importance of giving a sufficient amount of serum intravenously to effect the proper concentration of immune bodies in the blood. The authors of this article have been able to use antipneumococcus serum in a way to exhibit beneficial activity, if it possessed such a quality, inasmuch as in the cases treated a serum was used in repeated large doses intravenously, obtained from horses pushed to the highest possible degree of active immunity. In addition to this, it was known that the serum used in each case was active against the organism producing the disease in the individual. Both with the serum of Neufeld and Händel, which was univalent and known to be active against the commonest type of pneumococcus causing pneumonia, and with the authors' serum, a number of strikingly beneficial results have been obtained.

The degree to which antipneumococcus serum may be employed in the future must depend largely upon the constancy with which the serological groups of pneumococci previously mentioned are found. In the discussion of these organisms, it was shown that it would be impossible to treat cases specifically with sera against two of the groups, because in one of these groups the organisms are of distinct varieties, and the other does not yield a serum which confers passive immunity. The incidence of both groups is relatively small and infection with one of them rarely results in a fatal outcome. The other two groups, Groups I and II, as they have been designated, are the commonest types of pneumococcus met with in lobar pneumonia. Group I is responsible for about thirty-five per cent. of the cases ordinarily met with and yields by far the best protective serum which we have. Group II, on the other hand, yields a serum of considerably less efficiency. These latter cases are, in addition, among the most severe which are encountered. Further development in the methods of treating this latter variety of pneumonia is anticipated, and should the endeavors being carried on at the present
time prove successful, it would then be possible to treat effectively with specific antisera about seventy per cent. of all cases of lobar pneumonia. A method, at least, has been developed which makes the intelligent application of antipneumococcus serum possible, and it is to be hoped that with further studies of methods of increasing the potency of the serum obtained, successful results may be obtained in a constantly increasing number of cases of lobar pneumonia.

VACCINOTHERAPY

In turning from the question of specific serum therapy of pneumonia to vaccine therapy, which represents an attempt to stimulate to a point of increased utility those forces which the body is already marshelling to combat the disease, one feels the necessity of proceeding with considerable hesitation. The advance of serum therapy has in the main gone hand in hand with scientific advances in experimental methods made in the laboratory. Aside from the early studies of McDonald (44), who seems to have been able to induce artificial crises in rabbits infected with pneumococcus by the administration of a vaccine made from the organism with which the animal had been infected, but little laboratory work on the curative action of pneumococcus vaccine has been undertaken. In view of the rapidity with which rabbits develop a progressively increasing septicemia, even after subcutaneous inoculation with a virulent strain of pneumococcus, it seems unlikely that such results could be repeated with any constancy. For the most part the curative action of pneumococcus vaccine has been tested on human beings, and the reports of such attempts that have been published show, for the most part, an unfortunate lack of critical judgment. While in the main the mortality statistics seem to be good, so excellent in some cases that they approach the incredible, on the other hand, most of the evidence is impressionistic in character. Such objective signs of improvement as are possible of determination do not seem to have been sought for. In a number of instances, observations, having in view the changes in immunity in the vaccinated individual, were made on the opsonic index determined by the method of Wright. This method, even with other organisms, yields information of very doubtful value, and when applied to investigations of resistance to pneumococcus is admitted, even by Wright (81), when the usual technique is employed, to be of no real service.

The artificial production of an effective immunity against infectious diseases has been one of the most important problems to which investigators have devoted their efforts ever since the discovery of the causal relationship of bacteria to disease. In the field of animal experimentation the attempts have been rewarded with a large measure of success. To-day in the case of a large number of disease-producing micro-organisms, it
is possible to protect animals against infection by previously treating them with the same virus in some modified form. The adaptation of such methods to the prevention of disease occurring under natural circumstances has also been successful in a limited number of instances. Prophylactic vaccination against such typical infections as smallpox and typhoid fever in man and anthrax in animals has resulted in striking diminution in the incidence of these diseases whenever vaccination has been effectively carried on. In at least one instance it has been possible to prevent, by means of artificial immunization, the development of a disease after infection has occurred. The success of the antirabic vaccination of Pasteur with a modified rabies virus, has, however, no doubt been largely dependent upon the unusually prolonged incubation period of this disease. In cases where this incubation period is short, successful employment of the method of Pasteur is less common.

The extensive work of Wright (78) and his associates on the treatment of active disease by the use of bacterial vaccines has greatly stimulated the imagination, and, as a result, the activity of a large number of students of infectious diseases. A quarter of a century ago the procedure of injecting vaccines when the body is manifestly under the influence of the infecting agent would undoubtedly have been met with scepticism and failure. The successful immunization by Pasteur against rabies after the occurrence of infection, and in some instances even when symptoms were about to become manifest, and the apparent usefulness of Koch's tuberculin in certain cases of tuberculosis have led to a hopefulness which is still seeking justification. Wright's work on the treatment of local infections by suitable vaccines and the success which in many instances attends this method had added still further evidence in support of the procedure. The localization of an infection must, however, be regarded as the expression of a degree of immunity which is already moderately high. The great service of vaccines in this group of diseases lies in the fact that localized bacterial infections are exceedingly common, and represent in most cases an annoyance and an infirmity rather than a danger to life. In addition to these forms of infection, bacterial vaccines are now largely employed in conditions in which the specific agents of the disease can be detected in the blood, and in which the symptoms indicate that the infection is no longer strictly localized. They have been extensively employed in even such infections as typhoid fever, puerperal sepsis, general streptococcus infections, and in lobar pneumonia. Medical science unfortunately is unable to furnish an answer to the applicability of vaccines to the treatment of such infections. The appearance of such organisms in the blood in comparable infections in laboratory animals is usually rapidly followed by death. In many instances of such infections in man a like phenomenon is observed, so that it would seem from what we know of bacteriology and immunity that the employment of vaccines
in such acute conditions must have a very limited field. In spite of the presumptive evidence against the usefulness of vaccines in these diseases, the method has been widely favored, especially in the treatment of acute lobar pneumonia. It seems like adding fuel to the flames, but it may be that there are unknown factors in the path leading toward immunity that the bacteriologist has not yet discovered.

In studying the reports of the treatment of lobar pneumonia by means of pneumococcus vaccine, it is extremely difficult to arrive at a just estimate of the real value of the procedure. Many observers are unhesitatingly favorable in their impressions, and yet one feels that other investigators have arrived at contrary conclusions, or at least have failed to find sufficient evidence to support a general recommendation of the use of vaccines in this disease. Unfortunately many of these studies have failed to find their way into the literature of the subject, owing probably to a natural disinclination to report unfavorable results. This fact must be borne in mind then in the consideration of such reports as are available.

In America Stoner (69) has reviewed the results obtained from the treatment of one hundred and fifty cases of pneumonia by means of pneumococcus vaccine. These include cases treated by the following observers: 14 by Wolfe (77), of which 11 recovered, the death rate in the untreated controls being 40 per cent.; 13 by Boellke (10), with as many recoveries, the average duration of the disease after inoculation being 3 days; 83 cases by Leary (40), of whom 71 recovered, giving a death rate considerably below that ordinarily observed in untreated cases of pneumonia; 1 case by Batten (6) which recovered; 7 cases by Harris (30), 4 of which were benefited by the treatment showing an early crisis, and 3 which were not benefited; 1 case of delayed resolution by Allen (2) with recovery; 24 cases by Wilcox (75) with 23 recoveries, a truly remarkable result; 6 cases by Craig (14) with 6 recoveries; and 6 cases by Fisher with 5 recoveries. Of the 155 cases so treated, 135 cases recovered, showing a mortality of 13 per cent. Inasmuch as the average mortality statistics in pneumonia range from 20 to 35 per cent., these figures indicate a marked reduction in the death rate.

In Leary's 83 cases, 34 occurred in alcoholics, a class of patients in whom the death rate is usually high. Of these 34 cases but 6 died, a mortality of 17.7 per cent. Of the other 49 cases only 2 died, a death rate of 4.08 per cent., or a total mortality for the entire series of 83 cases, or 9.7 per cent. As far as one can determine in Leary's series of cases, autogenous vaccines were not used, and no mention is made of the source of the strains used or the care employed in their selection. Leary admits that his results are encouraging. In the eyes of the ordinary observer they are but little short of incredible. Stoner considers the 6 cases reported by Craig of particular interest. The patients were aged, 66 years, 67, 73, 75 years and 5 months, 80, and 83 years respectively.
Three of the patients were alcoholics and 2 of the cases followed an alcoholic debauch. Five had chronic nephritis and all had marked arteriosclerosis. All the cases were treated with vaccines and all recovered.

In Germany but little attention has been paid to methods of active immunization during the course of lobar pneumonia. Neufeld says that the outlook for favorably influencing an acutely progressive disease such as pneumonia, in which doubtless in all severe cases the infectious agent gains entrance to the blood, by means of subcutaneous inoculation of killed bacteria is very slight.

English writers accord more support to the method. Eyre (19), although he has had but little practical experience in the use of vaccines in pneumonia, favors their administration, and thinks that their beneficial action may be determined by their exhibition of a favorable influence on the opsonic index of the blood. He has found the use of vaccines especially valuable in the more chronic forms of pneumococcus infection of the lung. His opinion of the value of the opsonic index as a method for determining the degree of immunity was published some years ago and, in view of the more recent estimates of the serviceability of this method, may have been changed. Allen (1) is rather enthusiastic in his advocacy of the application of bacterial therapy to pneumonia. He emphasizes the importance of being sure that the pneumonia in question is due to pneumococcus before proceeding with the use of a stock vaccine. He prefers to use an autogenous vaccine when possible, and recommends the stock vaccine while the former is being prepared. In criticising adverse comment of certain other observers, he attributes their lack of a more signal success to the extreme rigor of their controls and a failure to use the vaccine in sufficient quantities. Morgan (47) has treated 43 cases with an autogenous vaccine with 2 deaths, a mortality of 5 per cent.; 1 of these died of nephritis after the subsidence of the pneumonia, thus reducing the mortality from the disease to 2.5 per cent. In many cases he employed repeated doses of 50,000,000 bacteria, but favors a somewhat smaller dose, 15- to 30,000,000. The temperature in some instances fell by artificial crisis and in others by lysis. From his experience Morgan thinks that the temperature may be a guide, but thinks the most noticeable feature of the treatment is the favorable change in the general condition without much change in the temperature. He does not think the opsonic index is a reliable method of estimating the progress of immunity in pneumonia, and admits the necessity of some means of determining whether or not any good effects develop which may be measured objectively. Harris (30) reports a number of cases in the same vein and thinks that the curative inoculation of pneumonia may be successful. He adds nothing in the way of determining objectively the amount of benefit derived. Both observers agree that the inoculations seem to do no harm. Char-
teris (12), on the other hand, reports 19 cases without any observable beneficial results.

Although many of the published reports indicate no small measure of success in the treatment of pneumonia with pneumococcus vaccine, one still feels unable to accord this form of therapy a recommendation for general application. Far too few attempts have been made to gain a solid foundation for the use of pneumococcus vaccine by means of scientifically conducted laboratory experimentation. The efficacy claimed is based entirely on mortality statistics and clinical impressions, supports which are well known to be notably misleading. With the exception of efforts of doubtful utility to correlate changes in the opsonic index of treated patients with the clinical course of the disease, practically no thought has been directed toward obtaining objective evidence of improvement, such as the disappearance of a bacteriemia or the appearance of readily demonstrable immune bodies in the blood. In many instances no attention has been paid to the existence of a multiplicity of races of pneumococci, and stock vaccines have been used consisting of strains about which nothing was known from an immunological standpoint. Such vaccines might easily contain only a single type of organism, or types which have no immunizing powers against the majority of types which ordinarily cause pneumonia. It is true that the best workers have sought to avoid such confusion by employing, whenever possible, vaccines made from the strain concerned in the particular case to be treated. No extended attempt has as yet been made to utilize the method of sensitization of pneumococci by specific serum antibodies in the treatment of pneumonia by pneumococcus vaccine. Levy and Aoki (41) have shown in animals that specific immune bodies appear in the blood considerably earlier when sensitized vaccines are used than when the animals are immunized by killed cultures not so treated.

From an experimental standpoint it is difficult to find support for the efficacy of methods designed to induce active immunization in such an acute and relatively short disease as lobar pneumonia. It is well known that in the active immunization of animals, antibodies do not appear in the blood in any considerable concentration much before the eighth or tenth day, the time at which an attack of pneumonia usually terminates naturally. Besides, it is difficult to see how the addition of small amounts of antigen could measurably affect the degree or quality of immune reactions in an individual who is only too often suffering from the presence of a superabundance of substances of like antigenic properties. If bacterial vaccines should prove of value in pneumonia before their efficacy can be generally admitted, at least some of these discrepancies must be eliminated.

Rosenow and Hektoen (65) have developed a modified vaccine for the treatment of pneumonia, prepared from partially autolyzed pneumococci.
They found that on suspending a virulent pneumococcus in salt solution, the substance on which depended its insusceptibility to phagocytic action was dissolved out. The soluble portion is toxic and not only has little immunizing properties, but even interferes with the formation of antibodies in animals. The insoluble remnants have well-marked antigenic qualities, and seem to be somewhat more serviceable in protecting animals than heat-killed suspensions of whole pneumococci. For these reasons they investigated the influence of virulent pneumococci, from which the toxic portions had been removed, on the course and death rate in lobar pneumonia. In different years the organisms were grown in somewhat different ways, and in the preparation of the antigens the cocci were allowed to autolyze in salt solution under certain conditions until most of them had become Gram-negative, a period at which they were usually sterile on cultural investigation. Some care had to be exercised to prevent the process of autolysis from going too far, because then all antigenic power might be lost. The dosage varied from 10- to 20,000,000,000, in some instances given once and in others repeated daily.

The cases treated were divided into three groups. The first group consisted of 30 cases treated by physicians outside of a hospital. The results in these were better than in the more unfavorable hospital cases. Of the 30 patients treated, 3 died. In the second group 35 cases occurring at the Cook County Hospital were treated. The mortality among these was 25.7 per cent. This is somewhat lower than the average mortality among cases of the same class. The third series formed much the largest group. In all, 294 cases are included in this lot, 146 having received injections of autolysed pneumococci and 148 serving as controls. No selection was practised, cases being taken alternately for injection and as controls. Of the 146 cases receiving injections, 34 died, a death rate of 23.3 per cent. Of the control group, 56 died, showing a death rate of 37.8 per cent. Comparing the two groups, one sees that in the injected series there was a lowering of the average death rate of 14.5 per cent. In view of the very bad type of cases treated, the test was a very severe one and the results are distinctly encouraging. Many of the patients were bad alcoholics, and numbers were first injected only after the disease had become well established. In general the results were better the earlier in the course of the disease the patient was injected. The injections in favorable cases usually provoked a slight rise in temperature, followed later by a drop, the temperature thereafter remaining at a somewhat lower level. Often if the injection was repeated at this point, the temperature reached normal in from three to five days after the onset. No harmful effects were noted in any case. The best results were naturally obtained in those cases treated outside the hospital because of the earlier period at which treatment could be begun. Of the cases treated in the hospital, among whom the results were not so good, the average
time of the first injection was the fifth day of the disease, necessarily a disadvantage in any form of treatment of pneumonia, and particularly for the methods under consideration. The incidence of complications and sequelae was about the same in both groups. In the injected series there was a tendency for the crisis to occur earlier than in the uninjected, especially where it was possible to start the injections early in the disease. In view of the fact that the mortality was consistently lower in the injected cases each year, that the average time of the first injection was late, and that the type of cases treated was of the worst kind, nearly one-half of the patients being bad alcoholics, Rosenow and Hektoen think that the conclusion is warranted that this method of treatment of pneumonia is of value.

From the experience of Wright, it would seem that pneumococcus vaccine might be used with advantage in the more chronic forms of pneumococcus infection of the lungs, such as delayed resolution and empyema. Indeed numbers of individuals have reported favorable results in such cases, but most of these represent isolated instances of such treatment, and no systematic study of its value in a large series of cases has as yet been carried out.

In recent years there has arisen in South Africa among the natives employed in the Rand mining district a severe type of pneumococcus pneumonia with a high death rate and incidence. In attempting to combat this condition Wright (79) has had an opportunity to test on a very large scale the value of prophylactic pneumococcus vaccination. After a considerable amount of experimentation, the administration of a single large dose containing 1,000,000,000 bacteria was found to be the best way in which to give the vaccine. Large numbers of natives running into the tens of thousands were available for the test. Every fourth individual failed to receive a dose of the vaccine, and these served as controls for the vaccinated. Careful records of the incidence of pneumonia among the vaccinated and unvaccinated were kept during a period of some months. Wright in his report of the work thinks that the prophylactic vaccination was effective in reducing the incidence of pneumonia among the natives during the first three months following inoculation. He was also able to treat with therapeutic vaccines quite a large number of natives after the development of the pneumonic process. His statistics of this procedure show practically no difference in the death rate between the inoculated and uninoculated. This he does not regard as indicating the inefficiency of the method, because the doses used were small. Another series of cases, inoculated with what he considered the optimum dose, and at a time that might be considered within the incubation period of the disease, showed a lesser incidence and death rate than the controls. Later reports of this work have failed to establish the efficacy of prophylactic vaccination in preventing the development of pneumonia, and
indicate approximately as high an incidence among the inoculated as among the uninoculated. Recent work (43) shows the existence of different races of pneumococcus from a serological standpoint as the infectious agent in pneumonia on the Rand, and, in the light of these studies, some improvement in the efficiency of the vaccine may be brought about by the use of special strains or strains to which the natives are exposed, factors that were not taken into account by Wright in his immunization experiments.

From this review, it is easily seen that the status of vaccination in pneumonia, both curative and prophylactic, is still very doubtful. In general, in infective processes associated with fever, science would forbid the use of such methods until it was determined whether or not the process represented a progressive undermining of the body resistance. In such conditions very small matters may influence the course of disease in an unfavorable manner, so that under such circumstances vaccination must be regarded as a highly experimental method, and should not be undertaken save under the advice of one trained in the problems of bacteriology and immunity. On the practical side the evidence of clinicians in favor of vaccination as a therapeutic measure in pneumonia is insufficient to overthrow the general scientific arguments against the procedure.

**LEUKOCYTE EXTRACT**

After consideration of the failure of immune sera and specific vaccines to influence favorably most diseases of infectious origin, Hiss (32) directed his attention to the important rôle played by the phagocytes in bacterial infection. He came to the conclusion that in recovery from many diseases we are dealing with an immunity which is largely cellular in type, not only in the sense of phagocytosis and digestion of bacteria, but also in the neutralization of poisons set free by their disintegration, the neutralizing bodies being contained largely within the phagocytic cells mainly for their own protection and not usually set free for the advantage of the cell community at large. This idea stimulated him to attempt to aid the leukocytes in their battle with the invading micro-organism by furnishing them as directly as possible with weapons to carry on the struggle successfully. These weapons, whatever might be their nature, he assumed might be furnished by an extract of the active substances of the leukocytes themselves, which were not ordinarily found free in the plasma. He considered that extracts would be more efficacious than living leukocytes themselves, since being diffusible they would probably be distributed impartially to all parts of the body and, as quickly as absorption would permit, relieve the fatigued leukocytes and protect, by any toxin-neutralizing or other power they might possess, the cells of highly special-
IZED FUNCTIONS. The extracts were prepared largely from leukocytes obtained from rabbits, were thoroughly emulsified in distilled water, allowed to stand at 37.5° C. for a few hours and then kept on ice until used. The total product, including residue and supernatant fluid, was used for injection. In addition to a number of other infections, these products have been used in the treatment of experimental pneumococcus infections in animals and in lobar pneumonia in man.

The animal experiments cited as a basis for the rationality of this form of therapy seem to indicate a favorable influence of the extracts on pneumococcus infection. Hiss says that in animals treated with the extract of leukocytes from normal rabbits, an infection, surely fatal in untreated controls, becomes markedly modified in such treated animals, even if the treatment is delayed many hours. Out of 8 control animals used in four experiments, in which the dose of pneumococci was the same, all died, averaging only 45 hours of life after being infected. Of the animals treated, some as late as 24 hours after infection, 9 out of 12 survived and 3 died with an average life of 60 hours after infection, 2 of which had not received treatment until after the expiration of 24 hours. A number of other experiments were performed in which the results were also favorable. On the other hand, living leukocytes introduced either subcutaneously or intraperitoneally, had no noticeable effect on like infections.

Encouraged by the results of these experiments upon animals, a limited number of observers have tested the efficacy of leukocyte extract in the treatment of lobar pneumonia in man. In 7 cases so treated, reported by Hiss and Zinsser (32), they thought that they observed a favorable action of the extract on the temperature and general condition of the patient, and a tendency for the number of leukocytes in the blood to increase subsequent to the injections. The leukocyte extract was given subcutaneously either in single or repeated doses of 10 c.c. Floyd and Lucas (21) have reported the treatment of 41 cases of pneumonia by the method of Hiss and Zinsser. Of these 41 cases, 5 died and 36 recovered, a mortality of 12 per cent. A comparison of 25 cases untreated with 25 treated cases shows a mortality more than double in the series of untreated cases as compared with the treated cases. Twelve of the cases were in children and in 29 the age ranged from 20 to 70 years. Their impressions were that in a number of cases the disease was appreciably shortened and with but few exceptions there was a noticeable improvement in the comfort and symptoms of the patient. In cases with severe toxemia, the effect of the injections was marked, and they feel that the agent may prove of considerable therapeutic value. The extract was given in doses of from 10 to 20 c.c., repeated from two to four times in 24 hours. In no instance did the treatments cause any ill effects.

Hiss (31) in a later paper gives an extremely favorable report of the
value of his method in the treatment of pneumonia. The total number of cases reported is 53. Of this number, 3 ended fatally, a mortality of 5.6 per cent. He says that the most obvious effects of the extract were an almost immediate improvement in the feeling of well-being of the patient, a beneficial change in the quality of the circulation and a reduction of the pulse rate. In some instances the crisis was early, and in others the temperature fall was by lysis. The spreading of the lesion was usually halted and the convalescence rapid and uninterrupted. One of the most notable effects was the increase in the leukocytosis that followed the treatments. His general conclusion is that in cases treated early the disease is rendered largely benign, and the course markedly shortened. In this series of cases the doses of extract employed were very much larger than those used previously, varying from 20 to 60 c.c. repeated every 4 hours.

In spite of the very favorable reports of the few observers who have undertaken to treat pneumonia by this method, it has not as yet received any wide application. From a theoretical standpoint it represents an attempt to supply a deficiency of a type of immune bodies which most observers believe to exist, and of which the importance is no doubt very great. Of their nature or mode of action, however, we know very little, and whether, when passed from one animal to another by means of artificial preparations, they are still effective may well be questioned. The work of Hiss indicates that this may be so, and from the clinical cases it would seem that the leukocytic substances of lower animals can stimulate a considerable degree of leukocytosis in man. The work deserves and requires further study before the results reported can be generally accepted.

CHEMOTHERAPY

To Morgenroth (48) and his assistants we owe the first progress that has been made so far in the attempt to control pneumococcus infection by means of a chemical compound with specific action. Because of the reports of the possible action of quinin in pneumonia, they used this alkaloid and substances closely related as a basis for their experimental observations on the effect of these substances on the course of experimental pneumococcus infections in animals. Morgenroth and Halberstaedter (49) had previously found that certain quinin derivatives were useful in the treatment of experimental trypanosomiasis and, because of certain characteristics which trypanosomes have in common with the pneumococcus, decided to test the efficiency of these bodies in pneumococcus infections. A number of derivatives, quinin, hydrochinin, hydrochloroquinin, ethylhydroquinin, and propylhydrocuprin, were employed in the experiments. The first positive results were obtained by Morgenroth
and Levy (48) by the use of ethylhydrocuprein. In their first experiments they employed a 25 per cent. watery solution of the drug and found, when this was injected into mice previous to injection of the infecting dose of pneumococcus, that whereas all the controls died, one-quarter of treated animals survived. This result is very striking, as virulent pneumococci injected into mice kill these animals with unfailing regularity. In curative experiments in animals injected with ethylhydrocuprein 6 hours after infection, 50 per cent. of the animals survived the controls. Under such conditions the administration of the drug undoubtedly effected a sterilization of the blood of the treated animals, inasmuch as in mice at such a period after infection with pneumococcus, septicemia has already developed. The drug was active not only against a single strain of pneumococcus, but also against many other strains of typical pneumococci.

Further studies by Morgenroth (50) and his associates showed that by modifying the technique of administration of the drug, still better results could be obtained. The toxic dose of this substance is but little above its curative dose. Injection of water solution allowed rapid absorption and this was not desired, as Morgenroth had shown that its action on the pneumococcus was best when it was continued for some hours. In order to obtain a like form of action in animals, the free alkaloidal base was injected in an oily suspension from which the rate of absorption was slow. When this was done, prophylactic experiments gave from 80 to 100 per cent. of survivals. In curative experiments the results were likewise improved by giving the drug in the same manner and repeating the dose every 24 hours for a few days.

Boehncke (9) has tested in animals the therapeutic activity of the drug when given in combination with antipneumococcus serum. Both in prophylactic and curative experiments the results were favorable, although the serum and drug were both used in quantities which of themselves were insufficient to bring about a favorable result. It is noteworthy that the disinfecting action of ethylhydrocuprein does not seem to be inhibited by the action of serum, as is the case in many such compounds. Boehncke found that in infections where he used mixtures of typical and atypical pneumococci, by repeated injections of the mixtures beneficial effects were observed, although the serum alone was completely inactive against the atypical races. Small doses of ethylhydrocuprein seemed to increase very much the efficiency of the antipneumococcus serum.

Moore (46a) in this country has carried on an extensive investigation of the action of ethylhydrocuprein or optochin, as it is more commonly called, against the pneumococcus. He has tested the bactericidal action of the drug in vitro against the different biological groups of pneumococcus and finds that it is equally active against all types, but that it possesses no such specific action against streptococcus. This investigator
has also found that the blood of rabbits after the administration of optochin, acquires bactericidal powers for pneumococcus. The best results are obtained by subcutaneous injection. It is somewhat less active in rabbits when given intramuscularly, and seems to exert no activity when administered by mouth. In order to obtain satisfactory effects by the intravenous route, it was necessary to give the drug in toxic amounts. Moore has also found that the blood serum of man becomes bactericidal for pneumococcus after the administration of 0.5 gram of optochin by mouth or subcutaneously. When given subcutaneously, the drug is very irritating and may produce necrosis with the formation of a sluggish ulcer. He has also tested the value of combining optochin with specific antipneumococcus serum in the treatment of pneumococcus infection in animals, and finds that doses of optochin, which in themselves are so small as to have no therapeutic value, enhance many times the protective value of threshold doses of antipneumococcus serum.

Parallel with the experimental work in animals on the efficiency of ethylhydrocuprein, observations on the efficiency of ethylhydrocuprein in the treatment of pneumonia in man have been carried on. Fraenkel (24) thinks that the drug is not yet suitable for human application, inasmuch as it has not a clear-cut action in a large proportion of cases. Wright (80) was unable to observe any therapeutic effects whatever. Unfortunately the toxic dose of the drug is so near the therapeutic dose that great care has to be taken in its use. Both noted several instances of ambylophia following its administration. Though the sight is recovered, it is possible that in some cases permanent blindness might result. According to Fraenkel the effects of the drug on the course of the disease were as follows: In all, 21 cases of pneumonia were treated with ethylhydrocuprein; in 9 of the cases treated, 43 per cent., there was no noticeable change following the exhibition of the drug; in 6 cases, 28.5 per cent., a doubtful result; and in 6 more cases, 28.5 per cent., a rather marked beneficial action. In the 6 cases in which the drug seemed to show some beneficial influence on the course of the pneumonic process, the temperature dropped off from the fourth to the fifth day. In 4 of the cases the temperature fell within 12 hours after the administration of the drug and in 2 it fell by lysis. The general character of the cases studied at this time was mild, and most of the patients recovered spontaneously.

Parkinson (58) has treated 9 cases of pneumonia with ethylhydrocuprein. Three of the cases had crises somewhat earlier than usual, the fourth to fifth day, but inasmuch as such early crises are not unusual, definite deductions cannot be drawn from them. Two patients died and in the remaining 4 the drug had no noticeable effect. Two of these later developed empyema. There was a slight rise in temperature following treatment in some of the cases, but no noteworthy effect on the pulse or respiration. In 3 cases out of the 9 treated, the pupils became widely
dilated, but there were no instances of amblyopia. His conclusions are that ethylhydrocuprein has no effect on pneumonia in man, and that toxic symptoms may appear after the administration of 1 gm. by mouth or 0.5 gm. hypodermically.

Baermann (4) has recently reported the treatment of 31 cases of pneumonia with ethylhydrocuprein, in some instances combined with serum obtained from patients convalescent from pneumonia. Of 5 cases treated by intramuscular injections of the ethylhydrocuprein base suspended in oil, favorable results were obtained in 3 cases, and 2 died. One of these latter had pneumococci in the blood and it is possible that the drug caused some diminution in their numbers. These patients all received repeated doses of 0.5 gm. ethylhydrocuprein suspended in oil, and no toxic effects are mentioned. Seven cases were treated with ethylhydrocuprein hydrochlorid by mouth in repeated doses of 0.25 gm. to 0.5 gm. No amblyopia was noted. Six of the patients so treated recovered and seemed to derive benefit from the use of the drug, and one died. Nineteen cases were treated by combinations of serum from convalescent patients and ethylhydrocuprein. In some the drug was given intramuscularly in oil suspension, and in others by mouth. Four of these cases died, and in the others the treatment in general seemed to be beneficial. In some instances pneumococci were found in the blood, and these either disappeared or diminished in numbers after the treatments. Baermann thinks that the drug has an unmistakable curative action in pneumonia and looks forward to further observations of its action, especially when combined with immune serum. His results seem to be distinctly better than those previously obtained, and may in part be due to better methods of administration.

The occurrence of the European war has delayed any increase in the general experience of the value of optochin as a method for treating lobar pneumonia. However, the drug has been rather widely used in Germany and a summary of the results obtained has recently been published by Leschke (40a). The cases are divided into two groups,—those treated before the third day of the disease and those treated after the third day. In the two hundred and four cases treated before the third day, the mortality was 5 per cent., and in the one hundred and nineteen cases treated after the third day, was 20 per cent. The mortality for the total three hundred and twenty-three cases was 1.1 per cent., which represents a considerable reduction in mortality from that ordinarily observed. Moore and Chesney (46b) have recently made a very careful study of the effect of optochin in lobar pneumonia, and also give a summary of the total number of treated cases up to the present time. In order to obtain an accurate knowledge of the use and effect of the drug, recourse should be had to the original article. These investigators recommend a dosage of the drug, based on body weight, of from 0.024 to 0.026 gram per kilogram, which
is the amount necessary to insure bactericidal development by the blood serum of the individual under treatment. An initial dose of 0.45 gram is given and the remainder divided into small doses of 0.15 gram given at from two to three hour intervals. The advantage of this method is that the bactericidal power of the blood rises rapidly and is maintained at a fairly constant level throughout the course of treatment. The administration of the drug is continued for about twenty-four hours after the temperature has fallen.

Optochin in certain individuals gives rise to toxic symptoms which constitute a distinct disadvantage in its use. The margin of safety is rather narrow and great care must be taken to avoid too large doses. The toxic effects seem to depend somewhat upon too great a concentration of the drug in the blood at one time and this is the reason for the repeated small doses, since when 0.5 gram doses are given the concentration rises rapidly and generally falls considerably before the time for the next dose. Optochin, like quinin, exhibits its chief toxic action against the special senses. Deafness not infrequently occurs during treatment, but recovery seems to be complete, and this is not necessarily regarded as an indication for the cessation of treatment. The effect of optochin on the eye, when given in toxic doses, is much more serious, and the administration of the drug should not be continued after the appearance of eye symptoms. These consist of widening of the pupil with failure to react to light, dimness of vision, and in some instances complete blindness. In extreme cases the eye grounds show pallor of the retina with marked narrowing of the vessels. Complete blindness may persist for a week or more, with gradual return of vision. In many instances recovery is complete, but in some there is apparently permanent damage to the retina so that although central vision is normal, there is marked contraction of the visual fields. In only one instance so far reported has blindness been permanent, a case in which a very large dosage of the drug was employed. Toxic eye symptoms, however, may develop after a comparatively small total dosage, 2.0 grams in 0.5 gram doses in one instance. Although such toxic effects are a serious disadvantage, there is reason to believe that by careful methods of administration they may be avoided or greatly minimized, and, in view of the gravity of pneumonia as a disease, it is to be expected that optochin will receive a wide application.

Recent investigations conducted by Lamar (39), though they do not belong to the field of specific chemotherapy, may be mentioned under this heading. The studies have in view the development of a method applicable to the treatment of localized pneumococcus infections, such as pneumococcus meningitis or arthritis. It was pointed out by Neufeld (54) some years ago that the pneumococcus is soluble in bile, very small amounts causing its complete disappearance. Certain other substances, whose physical action is much like that of bile, are known to exist.
most important of these are the unsaturated fatty acids. The soluble soaps of these acids, especially that of oleic acid, possess like bile the quality of dissolving pneumococci. Moreover, when pneumococci are exposed to their action, even in great dilution, they subsequently undergo autolysis much more rapidly and completely than organisms not so treated. Such soaped pneumococci when exposed to the action of normal serum disintegrate, but a few always remain and subsequently show active growth. On the other hand, when they are placed in antipneumococcus serum, the destruction is complete. The action of the serum is specific and shows no action against atypical strains that have been treated with oleate. It is known that considerable quantities of the unsaturated fatty acids exist in the animal cell, and are set free from the breaking down of the lecithin complexes when autolysis of tissue or resolution of lung occurs. The lytic action of these substances on pneumococcus is, however, suspended in the presence of protein-containing solutions, such as blood serum, so that their action in natural infection must be limited. Lamar was able to suspend the serum inhibition by adding to the soap serum mixtures an appropriate quantity of boric acid. Working with such mixtures he was able to obtain definitely beneficial results in local pneumococcus infections in animals. Infection could be prevented in small animals when the mixture was previously injected into the peritoneal cavity, infection following later in the same place. Therapeutic doses were also effective provided they were not given too long after infection had occurred. In a series of experimental pneumococccic meningitis in monkeys (38), treatment with soap, serum and boric acid mixtures showed very encouraging results. Infections of the meninges are especially suited to this method of treatment, because of the low protein content of the spinal fluid. In a number of instances Lamar was able to sterilize the spinal fluid of monkeys that had been infected some hours previous to the administration of the first dose of the therapeutic mixture. So far this method of treatment has not received any extended application to local pneumococcus infections in man, though it would seem well worth trying in such a hopeless condition as pneumococcus meningitis.

It has been suggested in the past few years by the advocates of camphor in the treatment of pneumonia that this substance has a direct action on the pneumococci. Boehncke (8) recently investigated this alleged action experimentally and found that he was able to protect animals against a fatal dose of pneumococci by treating them previously with varying doses of camphor in oil. He was unable to confirm Welch's (74) results on the therapeutic value of camphorated oil in rabbits when administered after infection had occurred. By means of large prophylactic doses, however, he was able to protect rabbits against surely fatal doses even when given intravenously. As in the case of ethylhydrocuprein, camphor was used by Boehncke in combination with antipneumo-
coccus serum. This method seemed to give better results than the administration of camphor alone. Camphor has been used, at times in large doses, for many years by physicians in the treatment of pneumonia, largely, it is true, as a circulatory stimulant, but it is likely that if it had any very marked specific action against the pneumococcus, this would have been noted.

**ULCUS CORNEÆ SERPENS**

Ulcus cornææ serpens is one of the severest types of ulceration of the eye. The process tends to spread rapidly and may involve considerable portions of the cornea. The process begins as a yellowish-gray infiltration near the center of the cornea. Ulceration rapidly takes place, the advancing edge becomes undermined and raised, the disease extends at the same time into the depths, so that perforation may quickly occur. There is almost always hypopyon, large amounts of the cornea may be destroyed, and occasionally panophthalmitis results. When healing occurs, all degrees of impairment of vision may result.

In about 98 per cent. of cases of ulcer serpens, the pneumococcus has been proven to be the etiological agent. As far as has been determined, the organisms found differ in no way from the varieties of pneumococcus causing lobar pneumonia in man. Römer (62) has devoted a number of years to study of the specific therapy of this affection. Experimental work has shown that immune bodies, either when produced actively or introduced passively by means of injections of specific sera, penetrate the cornea as well as other parts of the body, although in greatly reduced concentration. With these results as a basis, Römer and others have treated ulcer serpens with antipneumococcus serum. In animals prophylactic injections have prevented subsequent experimental infection of the cornea with pneumococcus. Römer's serum has been largely used in the therapy of human cases. It is prepared by the immunization of different animals to strains of pneumococcus obtained from cases of ulcer serpens, using preferably organisms of high virulence. The results, on the whole, seem to have been reasonably satisfactory and there seems to have been improvement from year to year. In favorable cases there is a reaction in the ulcer following the injection of serum, and this is followed by resolution. The extent of the process is much limited, and the hypopyon in many instances clears up as well. In general, the amount of permanent damage is much less in serum-treated cases than in those that are untreated. Paul (61), in a series of observations extending over a number of years, has had favorable results from the use of serum in 55 per cent. of his cases, and Gebb and Römer (28) in from 71 to 80 per cent. The outcome is more favorable the earlier the case is treated. When the ulcer is well
advanced, successful treatment becomes a much more difficult matter. Recently the best results have been obtained by the administration of a single large dose of antipneumococcus serum given either subcutaneously or intravenously. Although there have been a number of contradictory results, the weight of evidence indicates that antipneumococcus serum is a valuable aid in the treatment of ulcus serpens. As in pneumonia, the existence of different varieties of pneumococcus is probable, and the further adaptation of the serum to the types of pneumococcus concerned may increase its efficiency. In addition to the use of antipneumococcus serum alone, active immunization by means of vaccines, and a combined therapy using both vaccines and immune serum, have been employed in the treatment of ulcus serpens. Both methods have given some valuable results, especially the latter.

Since the introduction by Morgenroth of optochin into the therapy of pneumococcus infections, this drug has been used extensively in Germany in the treatment of ulcus serpens. Most of the investigators report satisfactory results from its application. A 1 to 2 per cent. water solution of the drug is applied locally and is said to result in unusually rapid healing of the ulcer. It causes no damage to the corneal epithelium in this dilution, and the burning sensation caused by its application can be obviated by the use of a local anesthetic. Pneumococcus ulcer of the cornea usually results in considerable destruction of tissue with scar formation. Treatment with optochin is said to give a more satisfactory end result as far as permanent damage to the cornea is concerned than any of the methods hitherto employed, especially those in which the cautery is used.

[Ophthalmologists in this country report distinctly favorable results from the treatment of pneumococcus ulcer of the cornea by local applications of optochin.—Editors.]

REFERENCES

2. ——. Lancet, 1909, ii, 780.
17. Dold. Das Bakterien-Anaphylatoxin und seine Bedeutung für die Infektion, Jena, 1912.
19. Eyre. Lancet, 1908, i, 539.
36. ——. Ref. Kolle-Wassermann’s Handb. der Path. microorg. 2d Ed., 1912, iv, 571.
44. MacDonald. Trans. Path. Soc. Lond., 1906, lvii, 45.
45. May. Münch. med. Woch., 1908, iv, 2083, 2140.
REFERENCES

64. ——. Jour. Inf. Dis., 1911, ix, 190.
79. ——. Lancet, 1914, cxxxvi, 1, 87.
80. ——. Lancet, 1914, ii, 1633, 1701.
81. ——. Lancet, 1914, cxxxvi, 1.
CHAPTER XIX

THE ETIOLOGY AND SPECIFIC TREATMENT OF RHEUMATISM

E. C. ROSENOW

GENERAL CONSIDERATIONS

The clinical evidence of the infectious character of acute rheumatic fever is quite convincing. The nature of the more chronic forms of the disease, however, and of the allied conditions such as chorea, rheumatic iritis, erythema nodosum, and erythema multiforme, is not so clear.

A large variety of organisms have been isolated from the lesions in rheumatic fever. Achalme (1), and later Thiroloix (22), and Triboulet (28) isolated from cases after death a large Gram-staining bacillus. Triboulet and Coyon (29) cultivated a diplococcus in five cases, pure cultures of which caused arthritis and endocarditis in rabbits. Two fatal cases showed the bacillus of Achalme in addition, and they concluded that the simple cases of rheumatism are due to the diplococcus alone, but that in the severe cases both organisms are responsible for the symptoms. It is now generally held that the bacillus of Achalme is identical with the bacillus Welchii (B. aërogenes capsulatus). Meyer (19) was the first to demonstrate that the streptococci associated with the relatively mild tonsillitis in rheumatism commonly produce arthritis, endocarditis, and pericarditis of the rheumatic type in rabbits. Riva (23), Beaton and Walker (3), Walker and Ruffel (31), Beattie and Yates (5), and especially Poynton and Payne (21) have isolated streptococci or diplococci quite uniformly from lesions in rheumatism after death, but only in a limited number of cases during life. These organisms closely resemble other streptococci morphologically and culturally, but when injected into animals produce effects quite different from those obtained by the injection of organisms coming from other sources than rheumatism. The experimental disease resembles very closely the disease in man. That rheumatism is a streptococcus disease is further indicated by the work of Tunnicliff (30) on the opsonic index; by the fact that the myocardial lesions produced experimentally by Coombs, Miller and Kettle (10) with streptococci from rheumatism are identical with those described by Aschoff
GENERAL CONSIDERATIONS

and Tawara (2) as characteristic of rheumatism in man; and by the fact that hemolytic streptococci, as shown by Jackson (14), and streptococcus viridans, as shown by Rosenow and Coombs (26), produce similar, although in the case of streptococcus viridans very much smaller, lesions in the myocardium of rabbits following intravenous injections of the respective strains.

The affinity for joints of streptococci from other sources than rheumatism has been emphasized by Cole (9), Meakins (18), Billings (6), Davis (11), Koch (16), Jackson (15), and others. The arthritis following injections of these streptococci is often monarticular, the exudate contains many cocci, suppuration and deformity occur, endocarditis and pericarditis develop only occasionally, while a grossly visible myocarditis has not been described. By injecting mixtures of hemolytic streptococci and streptococcus viridans Rosenow (24) has repeatedly produced arthritis and endocarditis in the same animal. The arthritis was proved to be due to hemolytic streptococci and the endocarditis to streptococcus viridans. But even here a gross pericarditis and myocarditis have not been observed.

A careful study of the protocols of the animal experiments performed by the investigators in these respective fields shows clearly that the results following injections of streptococci from rheumatism and streptococci from other sources respectively correspond quite closely to the differences observed clinically in artricular rheumatism, on the one hand, and streptococcal arthritis on the other. In spite of these facts, however, the view that rheumatism is due to a specific streptococcus, as claimed by Poynton and Payne, has not been generally accepted because so many investigators, notably Phillip (20), Cole (8), Beattie (4), Harrison (13), and others, have failed entirely, while others, as Loeb (17), have only rarely obtained the organism. And, finally, no one until recently has isolated the organism in a considerable number of consecutive uncomplicated cases of rheumatism during life.

During a study of pneumococci and streptococci I found that oxygen pressure evidently played an important rôle in bringing about the changes in these organisms, and it occurred to me that the peculiar distribution and character of the lesions as found in rheumatism might be due, among other things, to a great sensitiveness of the causative organism to oxygen, and that the negative results of cultures by so many investigators, particularly in the non-fatal cases, might be due to the failure in maintaining the proper oxygen tension in the culture media. Accordingly a technique was devised in which there is obtained, not only aerobic and anaerobic conditions, but a gradient of oxygen pressure between these extremes. This consisted of inoculating the joint exudate into tall columns of melted and cooled ascites-dextrose-agar. In this way I have isolated pure cultures of streptococci from the joints in 16 out of 19 non-fatal,
uncomplicated cases of rheumatism, and from the blood (by removing hemoglobin and complement with distilled water and planting the sediment as above) in 5 out of 8 cases. The negative results from the joints were obtained during convalescence. In 2 out of 4 cases the organism has been isolated from the stool during the height of the attack, and in one case from the tonsils. The aërobic and anaërobic cultures made in the usual way gave only a small number of positive results. No selection of cases was practiced except as to the time of making cultures. The organisms have been proved to be present in larger numbers in the periarticular structures than within the joint. In order to obtain positive results from the joint exudates it is necessary to make the cultures when the symptoms in a given joint are on the increase. It makes no difference whether the particular joint is the first to be involved in the attack or whether it is reinfected.

As further evidence that streptococci are commonly associated with rheumatism it should be stated that I have recently been able to isolate streptococci from the slightly enlarged but hyperemic extirpated lymph glands, draining the joints involved in 13 of 14 cases. At the height of the attack the B. Welchii was also found in two cases. In the glands the streptococci have been found during convalescence long after they have disappeared from the joint exudate. These results afforded opportunity to study the much disputed question whether or not the peculiar localization of the organisms in rheumatism was due primarily to peculiar properties of the streptococci or to peculiarities on the part of the individual infected, or to both factors. It is beyond the scope of this article to discuss in detail the cultural and other features of these organisms. It should be pointed out, however, that, although the strains isolated tended to fall in three distinct groups, all behaved differently in one respect or another from the usual streptococcus viridans and hemolytic streptococcus. They showed a relatively low but somewhat wide range of virulence; a capacity to produce much acid in media containing dextrose; a rather marked tendency to change in properties on cultivation; ability to grow better than other streptococci at a low temperature; and a relatively higher virulence than pneumococci and streptococci for cold-blooded animals (frogs). By inoculating rabbits and dogs as soon as an abundant growth could be obtained the chief point of difference from streptococci isolated from the usual sources was shown, not only by their simultaneous affinity for the endocardium, pericardium, myocardium, and joints, but by their localization in the animals in sites corresponding roughly to those in the case from which they were isolated. Thus the strains isolated from cases without muscle involvement never produced muscle lesions, but endocarditis and arthritis, and sometimes pericarditis, while those strains isolated from the lesions in the muscles in man, as well as those isolated from the joints in cases showing definite muscle involvement, produced, in addition to endo-
carditis and arthritis, a non-suppurative myositis and a pronounced myocardiitis. The strains from cases in which both endocarditis and pericarditis were present were the most virulent, and these showed greater tendency to infect the pericardium of rabbits and dogs than the other strains. After cultivation for only a short period, and after repeated passage through animals, they lose their affinity for special structures in the experimental animals. The endocarditis produced by injections of these strains into rabbits and dogs has been proved to be embolic in origin, and in both species the type of lesion produced corresponds quite closely to that described as characteristic of rheumatic endocarditis in man. The infection involves most frequently the mitral valve and chordae tendineae, tends to remain subendothelial, or to produce small warty vegetations, and shows a marked tendency to heal, leaving a nodular scar. This is in marked contrast to the type of endocarditis produced commonly by streptococcus viridans; in the latter, as in chronic infectious endocarditis in man, the vegetations grow large, ulcerate, and produce the fatal form of the disease.

The close relation which exists between rheumatism and chorea in man is paralleled to a degree by the animal experiments. Five half-grown rabbits and two white rats, all of which were injected either with strains from rheumatism after one animal passage or with one strain of a streptococcus possessing similar properties, showed symptoms of meningeal irritation, although there were no typical choreic movements, and localized grayish-white nodular areas in the pia mater. That chorea is due to a streptococcus having peculiar properties has recently been suggested by Dick and Rothstein (12).

Experimental evidence has been produced which goes to show that probably lodgment in the fine capillaries of the iris occurs in rheumatic iritis, and that muscular rheumatism, or rheumatic myositis, is commonly due to streptococci closely related to those found in articular rheumatism. The rather striking affinity shown by these strains in my experiments for the appendix (as found also by Poynton and Payne, 21), the diarrhea due to colitis, and the enlargement of the mesenteric lymph nodes observed commonly in animals after intravenous injections, as well as the isolation of the organism from the stool during rheumatism in man, indicate that the organisms may gain entrance through the lymph structures of the intestinal tract. It must not be supposed, however, that the streptococci associated with rheumatism are a group of organisms far removed from other streptococci, or that they are necessarily specific for rheumatism. By appropriate means I (25) have converted these strains into the other members of the streptococcus group, and have made other streptococci acquire the features of the strains from rheumatism. These facts are good evidence against the view held by some that the streptococci found in the joints in rheumatism are merely secondary invaders, and that the
real cause of the disease is still unknown. The experiments on mutation show further that when these and other streptococci are grown in symbiosis with other bacteria and under a varying degree of oxygen pressure they may acquire new features. The places in the human body where such conditions prevail, and where special features are likely to be acquired, are foci of infection such as occur in the tonsils, various sinuses, the appendix, and infections about the teeth and gums. That changes in the character of the bacteria actually occur in the tonsils in rheumatism seems quite clear; the mild character of the tonsillitis at the time of the attack, and the late appearance of rheumatism in some cases of acute follicular tonsillitis (streptococcal), support this idea. The focus may be unusual. Thus in two cases of rheumatism in young men 20 and 35 years of age, both of whom developed pericarditis and endocarditis, the attacks came on without an associated tonsillitis ten days after a crushing injury to the thumb in one instance, and after an indefinite period of infected ingrowing nails of the great toes in the other. Streptococci were isolated repeatedly during the attack from these regions in both, and the disease, including pericarditis, was reproduced in rabbits and dogs.

[The acquirement of new characters by organisms seems to be an occasional occurrence, but a frequent change in morphologic characteristics does not seem to be in keeping with other facts of biology or bacteriology. When organisms do undergo changes these changes are perhaps more likely to concern physiologic characters, such as the fermentative reactions, than they are to involve changes of morphology. The conditions required by experimentation along this line are exacting, and one must be extremely cautious in the interpretation of results which seem to indicate sudden accession or loss of biological characteristics.—Editors.]

While it is true that acute rheumatic fever has a distinct clinical picture, it is equally true that certain cases, especially the more chronic form of the disease, cannot be differentiated from the early stages of some cases of arthritis deformans. Again, certain cases of chronic infectious endocarditis follow in the wake of an acute rheumatism.

Recently I have had opportunity to study the streptococci isolated from the glands draining the involved joints in arthritis deformans, and find that these organisms have distinctive cultural and pathogenic features which are in keeping with the type of disease produced in man. Moreover, each of the strains of streptococci which I have isolated from the blood during life in four cases presenting the typical clinical picture and post-mortem findings of chronic infectious endocarditis, but which followed attacks of acute rheumatism, had the cultural and pathogenic features of streptococcus viridans isolated from other cases in which there was no history of rheumatism. From these facts it is clear that the character of the disease produced, and the location of the lesion, depend to a very large degree upon the character of the infecting streptococcus. There is,
on the other hand, good evidence that peculiarities of the infected individuals, especially those liable to repeated attacks of rheumatism, play a rôle. For some reason these individuals seem to be susceptible or react peculiarly toward this group of streptococci or otherwise furnish conditions such as foci of infection which favor the entrance into the body and the acquirement of the special features by these organisms.

SPECIFIC THERAPY

The study of a large series of strains of these organisms indicates that a rational specific therapy for rheumatic fever must take into account the fact that, while the causative agent in at least most cases of rheumatism is a streptococcus, the various strains differ from each other in cultural features, in degree of virulence, and especially in their affinity for various structures when injected in animals. These facts must be borne in mind in active immunization, and may explain why salicylic acid, for example, acts as a specific in certain cases, especially in early acute cases, while others are apparently not benefited; and why cacodylate of soda given hypodermically in large doses has little or no effect on the joint symptoms, but in the presence of a pericarditis has a marked beneficial action. The unfavorable results from the use of anti-streptococcous sera are best explained by the fact that the streptococci from which they are prepared are not like those from rheumatism. The objection which may be raised against the usual heat-killed vaccines for rheumatism now on the market is that they are prepared from strains of streptococci from the tonsils in rheumatism, which may or may not be the causative organisms, or from strains isolated from the joints or blood after long cultivation on artificial media, and hence probably after they have lost the properties essential to an efficient antigen. Soon after the strains from joints were isolated in 1911 in a series of cases of rheumatism I prepared a large quantity of vaccine containing a mixture of 12 strains. This was done by growing them in ascites-dextrose-broth and then suspending them in NaCl solution under ether at 37° C. until all organisms were dead, and until a large portion had become Gram-negative. The toxicity of the suspension for guinea-pigs was proved to be slight. This vaccine was then used in conjunction with Dr. Falls for the treatment of cases of rheumatism. Sixty cases which were suffering from acute or chronic rheumatism were treated. Approximately 2,000,000,000 of the partially autolysed organisms were injected daily for three or four days, and then at longer intervals. In most of these cases there seemed to be a beneficial action; in some it was slight, while in a few cases no beneficial action could be made out.

[We have seen no permanent benefit ascribable to vaccines alone, following their use in cases of this sort.—Editors.]
REFERENCES

5. —— and Yates. Jour. Path. and Bact., 1912, xvi, 246; 1913, xvii, 538.
15. ——. Jour. Inf. Dis., 1913, 12, 364.
22. —— and ——. Summary of various papers in book form, Researches on Rheumatism, 1913. The Macmillan Co., N. Y.
27. Thiroloix. La sem. méd., 1896, 376 and 420; Compt. rend. de la soc. de biol., 1896, 268.
CHAPTER XX

EPIDEMIC CEREBROSPINAL MENINGITIS

A. SOPHIAN

INTRODUCTORY

The broad term meningitis indicates an inflammation of the meninges, the causes of which are many.

The causes may be divided into the bacterial and non-bacterial. The bacterial infective group produces a suppurrative inflammation, and includes the following group of bacteria: the meningococcus, influenza bacillus, tubercle bacillus, streptococcus pyogenes, streptococcus mucosus capsulatus, pneumococcus, and staphylococcus; less commonly the typhoid bacillus, colon bacillus, the bacillus of bubonic plague, of glands; the bacillus pyocyaneus, the gonococcus, and micrococcus tetragenus. In this group may properly be included poliomyelitis and its various subdivisions and syphilitic meningitis.

The non-bacterial division of meningitis includes a small group named aseptic meningitis and a larger group called meningismus.

Aseptic meningitis is a suppurrative meningitis not directly incited by any bacteria, but rather produced by a suppurrative inflammation of tissues contiguous to the meninges; this most often refers to inflammation of the skull and its various sinuses, e. g., complicating frontal sinusitis; in severe middle-ear infection, and infections of the cavernous and other skull sinuses.

The group classed as meningismus is of very common occurrence. Meningismus is an inflammation of the meninges occurring during the course of general septicemic infections, resulting principally from the general toxemia which complicates and is part of the disease. It is most often seen in bronchopneumonia in young children, particularly in the form with extensive apical consolidation, and quite often in typhoid fever during the second and third weeks. The condition is essentially a toxic irritation of the meninges. No gross macroscopic changes can be found in the meninges post mortem, though some changes have been found in the membranes by careful microscopic examination.
To summarize, meningitis is an inflammation of the meninges, which may be of infective origin, produced by any of the known pathogenic bacteria, or toxic irritative in origin, occurring during the courses of general bacterial infection, or complicating severe toxemia from any other cause.

The classification of meningitis may be further simplified by dividing the condition into primary and secondary meningitis.

Primary inflammation of the meninges may be produced by any of the following bacteria: the meningococcus, influenza bacillus, tubercle bacillus, and streptococcus mucosus capsulatus.

Primary meningitis caused by the tubercle bacillus very occasionally occurs, but infection undoubtedly is almost always secondary. We may, therefore, eliminate this germ from the classification.

Similarly, primary influenzal meningitis and streptococcus mucosus capsulatus meningitis, while occasionally seen, are practically unknown in epidemic form. These may also, therefore, be eliminated from important consideration in this group.

Meningococcic or, as it is generally styled, epidemic meningitis is the most important form of primary meningitis. It is the form which has caused large and repeated epidemics, and is most important from a therapeutic standpoint on account of its frequency and the high rate of mortality when not treated by specific measures.

Under the secondary form of meningitis may be grouped the other pyogenic forms of meningitis and meningismus. They all occur as a complication secondary to some other infection; thus streptococcic meningitis, as a rule, is secondary to streptococcic middle-ear infection; staphylococcic meningitis occurs secondary to general staphylococcic bacteriemia following some local staphylococcic infection either of the bones or infection of the soft parts. Meningismus, as has been explained, occurs secondary to some general systemic infection, usually one of the group of acute infectious diseases.

In encountering a case with symptoms of meningitis, therefore, the first and most important consideration is to determine with which form of meningitis one is dealing, and, if bacterial, whether it is due to the meningococcus or whether it is the secondary type of meningitis caused by some of the other bacteria cited.

The general clinical symptoms of all forms of meningitis are similar. A careful study of the history and onset of the disease, the grouping of symptoms, the diagnosis of some other primary infection as typhoid, pneumonia, middle-ear infection, or some other local infection, are undoubtedly of considerable importance in determining the type of meningitis from which the patient is suffering.

There is only one way to prove, however, whether a case of meningitis is infective or toxic in origin and to establish definitely the bacteriological type of the infection. An examination of the cerebrospinal fluid will, as
a rule, clear up the diagnosis. It will furthermore materially aid the more accurate diagnosis of infantile paralysis and syphilitic meningitis, and will help to establish the diagnosis of toxic meningitis (meningismus).

During the course of an acute infectious disease, like pneumonia, it is sometimes very difficult, by clinical methods alone, to prove definitely whether complicating symptoms of meningitis are toxic and due to the original infection or whether the symptoms of meningitis are due to a coincident attack of pneumococcic, meningococcic, or other bacterial form of meningitis. Lumbar puncture with examination of the cerebrospinal fluid will readily differentiate the pathological condition.

It may be well in these pages to outline the laboratory findings in the cerebrospinal fluid in the various forms of meningitis, since upon this important step depends the application of the active curative, remedial measures.

TECHNIQUE OF EXAMINING THE CEREBROSPINAL FLUID

In examining the cerebrospinal fluid the following important data should be carefully noted: the pressure of the fluid as it flows from the needle, its color and turbidity, the presence of fibrin in the fluid, the cytology and bacteriology. In certain instances it will be necessary to make special serological tests and to inoculate animals with the fluid.

**Pressure of the Cerebrospinal Fluid.**—Special instruments have been devised to determine the cerebrospinal fluid pressure, the principle in all being to measure the height to which the fluid will rise in a glass tube which is connected to the needle. Some use bent tubes; others, straight tubes; some, graduated; others, ungraduated. The height proper is ascertained by means of a tape measure. The bore of the tubing used by all approximates 1 mm. In terms of water pressure the normal cerebrospinal fluid pressure has been determined to be from 60 mm. to 130 mm. In a sitting posture the pressure is much higher.

It is unnecessary in most instances to take special measurements of the cerebrospinal fluid pressure. A normal cerebrospinal fluid flows from the needle very slowly, averaging about one drop every 3 to 5 seconds. In the various forms of meningitis, depending upon the amount of pressure and hydrocephalus, the fluid flows from the needle very much more forcibly, very often in a continuous stream. Thus, at a glance, one can readily determine whether one is dealing with a normal condition or with severe or moderate hydrocephalus.

The pressure of the cerebrospinal fluid in epidemic meningitis varies very considerably in different cases and at different stages of the disease. Early in the disease it is often only moderately increased, averaging not much over 150 to 300 mm. Late in the disease with the establishment of
a chronic severe hydrocephalus in cases where there is free communication between the ventricles and subarachnoid space, the pressure is often very great, running up from 600 to 800 mm.

Careful observation of relative cerebrospinal fluid pressure at different lumbar punctures during treatment of a case of epidemic meningitis often gives an important clue as to the progress of the disease and the treatment that should be employed.

**Color of the Cerebrospinal Fluid.**—A normal cerebrospinal fluid is clear and colorless. Tuberculous meningitis, except in rare instances, the various forms of syphilitic and parasyphilitic meningitis, poliomyelitis, and polioencephalitis yield a clear fluid containing fine flocculi. Epidemic meningitis and the other supplicative forms of meningitis yield a turbid fluid, the degree of turbidity usually depending upon the degree of infection.

**Fibrin Content.**—The microscopic fibrin formation can be readily determined in a normal fluid if, after removal, the fluid be permitted to remain undisturbed for a few hours. In most pathological fluids a fibrin network forms or clumps of fibrin settle upon standing.

**Chemical Examination of the Cerebrospinal Fluid.**—A normal cerebrospinal fluid contains very little protein. In all inflammatory conditions of the subarachnoid space and ventricles, as a direct result of the inflammation, there is an increase of protein content in the cerebrospinal fluid.

The cerebrospinal fluid may be increased in quantity and pressure by causes other than infectious: (1) in tumor of the brain; (2) in cardiac and kidney incompetency with general anasarca; (3) in the meningismus form of irritation from any of the causes referred to; (4) in general convulsions in children from causes other than disease of the central nervous system; (5) in temporary hydrocephalus from severe headache and occasionally following the use of drugs. In these conditions, all of which may be accompanied by symptoms of headache, vomiting, vertigo, and other symptoms suggestive of meningitis and sometimes indicating a lumbar puncture, the cerebrospinal fluid examination for its chemical content will readily differentiate between the increase of the cerebrospinal fluid of non-infective origin and the true infective, inflammatory meningitis.

All of the tests described are concerned with the precipitation of protein by chemicals. A simple test is the layering of pure nitric acid over the cerebrospinal fluid, the appearance of a cloud at the junction indicating a positive reaction. A similar one consists of the addition of a few drops of 5 per cent. acetic acid to a few cubic centimeters of fluid, likewise causing the appearance of a white precipitate when positive.

A somewhat more delicate test is the Nonné test. This is divided into two phases, the first being obtained by adding saturated ammonium sulphate solution to cerebrospinal fluid in equal parts. This precipitates the
globulin. After three minutes an estimate should be made of the degree of reaction. All fluids, including the normal, yield a cloud in this phase. In the second phase the mixture is filtered, and to the filtrate is added one drop of dilute acetic acid, and the solution is boiled. The appearance of a cloud is believed to be due to a serum albumin of inflammatory origin, and is considered a positive reaction.

Another test of equal delicacy is Noguchi’s globulin test. This is performed by mixing one part of cerebrospinal fluid with five parts of 10 per cent. butyric acid in physiological salt solution, boiling, then quickly adding one part of a normal solution of NaOH and boiling again for a few seconds. A normal fluid produces a slight, white, diffuse cloud that does not precipitate. An exudate from inflammatory meningitis produces a heavy white cloud that precipitates in the form of large flocculi. Noguchi advises that a fluid should be allowed to stand from at least one-half to one hour before readings are made.

Another test, which in the writer’s experience has not been of as great help as the others, described by Braun and Husler (4), consists of the addition of 1 c. c. of cerebrospinal fluid to n/300 HCl and slowly shaking. If clouding does not occur after 5 c. c. have been added the reaction is considered negative. Sometimes a positive reaction does not occur for one-half hour.

The gold chlorid test and other tests, all of which are concerned with the chemical precipitation of the albumins and globulin, have been used. The very simple acetic acid and the nitric acid tests are almost of as great significance as the more complicated tests recommended.

Another chemical means recommended for differentiating between inflammatory fluids, normal fluids, and transudates is the reduction of Fehling’s solution by the cerebrospinal fluid. A normal fluid reduces Fehling’s solution after the addition of a few cubic centimeters of fluid. Most observers believe that purulent fluids and fluids of tuberculous meningitis do not reduce Fehling’s solution. It is true that gross reduction does not as readily occur in purulent fluids and fluids of tuberculous meningitis as in normal fluids, but upon adding a sufficiently large quantity of fluid in any inflammatory condition and boiling with Fehling’s solution a reduction sediment can in many instances be determined if the fluid be allowed to settle for a few hours before examination. In the writer’s experience this test is of little, if any, significance.

To recapitulate: It must be borne in mind that the chemical examination of the cerebrospinal fluid for reduction of Fehling’s solution and the presence of protein content are not of definite diagnostic significance, being of value only in differentiating grossly normal fluids and transudates from the fluids in inflammatory meningitis, whatever the cause.

**Bacteriology.**—This is the most important examination of the cerebrospinal fluid and one that can easily be employed in the office of the gen-
eral practitioner. The technique is as follows: Fluids should be centrifuged for several minutes until a moderate amount of sediment is collected. The supernatant fluid should be poured off and used for the chemical tests. A little of the sediment is smeared on a glass slide and stained with Gram's stain. If influenzal meningitis be present the sediment should be stained with fuchsin, as sometimes the influenza bacilli may be missed with simple Gram stain. If bacterial, suppurative meningitis be present the causative bacteria can be readily demonstrated in moderate or large numbers in most instances. If tuberculous meningitis be suspected the fluid should be centrifuged for a longer period, from one-half to one hour, and the greater part of the sediment should be smeared over a cover slip and allowed to dry. A part of the fibrin network should be fished out and streaked over the same slide as the sediment. After drying the cover slip or slide should be stained with the regular Ziehl tubercle stain; tubercle bacilli, in the great majority of instances, will be found, though few in number, after a patient search.

In the usual pyogenic meningitis, after a loopful of sediment is taken from the smear, several loopfuls should be streaked on suitable culture media, the most favorable media being that containing the usual nutrient agar mixed with 1\(\frac{1}{2}\) per cent. glucose and 1/6 the volume ascitic fluid or sterile animal serum. After incubation for 18 to 24 hours at 37° C. a growth usually appears, though sometimes in influenzal meningitis the growth is delayed for 3 to 4 days. Gram's stain of this growth and the morphological appearance of the growth will usually enable positive diagnosis at this time. Further cultural identification of the growth must, of course, be made when necessary. In meningococcus meningitis the findings are usually typical. The Gram-negative biscuit-shaped diplococci, extracellular and intracellular, the irregular staining of the cocci, their frequent clumping, the typical appearance of the colonies, the tendency to rapid autolysis of the germ in culture media and in salt solution permit of diagnosis within a very short time.

Cytology.—Careful cytological examination of the cerebrospinal fluid will yield considerable information of great diagnostic importance. The method consists in determining the total number of cells in the cerebrospinal fluid and in differentiating the type of cells. The simplest method employed is that in which the cerebrospinal fluid after its removal is centrifuged and the sediment poured on a slide as for the bacteriological examination; after staining, the number and type of cells as they appear in the smear are determined. A normal fluid shows an occasional endothelial cell or lymphocytes in the field. In all forms of inflammatory meningitis the cells are considerably increased in number. The type of cells depends upon the character of the inflammation. In purulent inflammation, due to the usual pyogenic bacteria, such as meningococcus, streptococcus, pneumococcus, and the others, the bacteria are almost wholly pus cells, poly-
mophonuclears. In tuberculous meningitis, syphilitic and parasyphilitic meningitis, poliomyelitis, and polioencephalitis the cells are usually lymphocytes.

More accurate methods for determining the number of cells have been devised and used. The principle in these methods is the actual counting of the cells on a blood-counting slide. Some workers centrifuge the fluid and study the sediment on a blood-counting slide, while others recommend the use of a staining fluid which should be mixed with the cerebrospinal fluid immediately after removal, and then the cells counted in the regular counting chamber as for a blood examination. The staining solution commonly used consists of the following:

<table>
<thead>
<tr>
<th>Methyl violet</th>
<th>Glacial acetic acid</th>
<th>Distilled water to make</th>
</tr>
</thead>
<tbody>
<tr>
<td>............................</td>
<td>............................</td>
<td>............................</td>
</tr>
<tr>
<td>1</td>
<td>2.0</td>
<td>50.0</td>
</tr>
</tbody>
</table>

This solution is drawn up into an ordinary white-blood-counting pipette to the .5 mark and the cerebrospinal fluid drawn up into the diluting chamber as for a regular blood count. Either a regular blood-counting chamber may be employed or special chambers which have been devised.

A normal cerebrospinal fluid contains on an average of 7 to 10 cells per cubic millimeter. In inflammatory meningitis the cells as mentioned are considerably increased up to several hundred cells per cubic millimeter.

The above-described examinations constitute the usual studies of the cerebrospinal fluid. In suspected tuberculous meningitis, even where the bacilli have been found in the cerebrospinal fluid, a few cubic centimeters should be injected into a guinea-pig. If positive, the guinea-pig will usually develop miliary tuberculosis in four to six weeks.

Serological studies of the cerebrospinal fluid are only of academic interest, but are not of immediate practical application.

In the premeningitic stage before the full establishment of the symptoms of meningitis the cerebrospinal fluid is increased in quantity, clear, sometimes showing a slight increase in fibrin and a faint increase in the total protein content as demonstrated by the chemical tests previously described. Cytology shows either no increase or a moderate increase in cells, the latter most often being lymphocytes. Sometimes these cells are equally divided between lymphocytes and polymorphonuclear cells; at other times polymorphonuclear cells predominate. As a rule, however, early in the premeningitic stage, lymphocytes are more numerous. As this stage merges into the true stage of meningitis polymorphonuclear cells are in excess, and in the true stage of meningitis polymorphonuclear cells practically exclude all other types of cells.

The stained sediment of the fluid in the premeningitic stage shows most often no bacteria or may exhibit a few free Gram-negative diplococci. These are evidence, and are part of the general bacteriemia rather
than an indication of the localization of the organism in the meninges. Late in this stage of the disease the organisms are more numerous and free, and then indicate the beginning of the localization of the meningococci in the meninges. Culture early in this stage when the organisms are few is, as a rule, negative. Late in this stage it is usually positive, showing after 18 to 24 hours' incubation the usual characteristic growth of meningococcus.

In the fully developed case of meningitis the cerebrospinal fluid usually shows the following classical findings: a turbid fluid from slightly opalescent to thick, viscid, plastic pus usually under high pressure and markedly increased in quantity; at times 100 c. c. or more fluid may be easily removed. Fibrin and protein content is very markedly increased. A study of the sediment demonstrates a very pronounced increase in cellular elements, practically all of the cells being polymorphonuclears. The stained smear usually exhibits varying numbers of Gram-negative diplococci, both extra- and intracellular. In severe cases, before serum treatment, or in cases which are not responding to serum treatment, most of the bacteria are extracellular. With favorable response to serum treatment, or in cases that are doing well without serum treatment, the bacteria are fewer in number, most being intracellular. With favorable response there is often a tendency for the organisms to clump. The bacteria ordinarily stain very irregularly in smear, some taking a deep stain, others being mere shadows. There is often a tendency for the bacteria to rapidly diminish in numbers after the disease has lasted only a short time, even if there be no improvement in the clinical condition or if the disease be aggravated. In this instance, however, the bacteria, though few, are almost altogether extracellular.

In the chronic form of meningitis the cerebrospinal fluid findings vary, depending upon the type of infection. In the severe form of the disease the findings are exactly the same as in the usual acute form of epidemic meningitis except that the pus cells are less numerous and lymphocytes abound in larger proportion. The longer the case lasts the greater the tendency for the percentage of lymphocytes to increase and the percentage of polymorphonuclear cells to diminish. If serum treatment be instituted in these cases, even if there be no improvement, there is generally a prompt change in the cytological picture. Polymorphonuclear cells promptly increase, and may entirely replace the lymphocytes. In the mild form of the disease the fluid is usually only slightly opalescent and very markedly increased in quantity; fibrin and protein content is moderately increased; the number of cells is moderately augmented, the lymphocytes being equally divided with the polymorphonuclear leukocytes; the bacteria, often clumped and intracellular, are usually very few, very often a few also being extracellular. If serum treatment be introduced, and if there be response, the cells, mostly polymorphonuclears, increase considerably in number.
TREATMENT OF EPIDEMIC MENINGITIS

With this the few extracellular bacteria become intracellular, and with further treatment the bacteria totally disappear.

TREATMENT OF EPIDEMIC MENINGITIS

The present recognized treatment of meningitis is one of the great scientific achievements of the twentieth century. It was brought about by a very careful study of a number of important factors: the bacteriology of the meningococcus; the recognition of the pathological sequence of the meningococcus infection, and the recognition of the fact that the meningococcus infection is first a violent bacterial infection, which begins as a severe, general meningococcic bacteriemia that only later is followed by an infection of the cerebrospinal meninges.

It was learned that sometimes a patient dies from the severe general bacteriemia even before the infection localizes in the meninges. With localization in the meninges the disease, to a very great extent, becomes a local one, the general sepsis, as a rule, abating or dying out.

The treatment after meningitis sets in resolves itself, as in other local infections, into combating and destroying the infectious agent and relieving the immediate urgent symptoms resulting from the local multiplication of the infectious agent. The treatment thus consists of specific serum therapy for the infection and the removal of the exudate caused by the infection. In all inflammations of the meninges this is most important on account of the hydrocephalic symptoms resulting from the confinement of the exudate in the meninges, which are bounded on one side by the bony skull and on the other side by the softer brain tissues. As the fluid collects in larger quantities pressure is thus exerted on the important centers within the brain.

The first advance in this field of study was the preparation of a specific immune serum. This was done almost coincidentally by Flexner (7) in this country and Jochmann (11), Kolle and Wassermann (12) abroad. All investigators worked practically along the same lines, and attempted to produce a serum of high opsonic, bactericidal, and antitoxic properties.

Similar methods were employed by all. At first smaller animals (the rabbit) were used; later larger animals were immunized—the goat, sheep, monkey (by Flexner), and finally the horse. The methods used and generally now accepted are as follows: (1) injections of dissolved meningococci (so-called meningococcic extract) in increasing quantities, followed by injection of live culture in increasing quantities. The object of the former is to produce a serum of high antitoxic potency and the latter to produce a serum of high opsonic and bactericidal properties. Horses are now generally used. After two months a horse, as a rule, can endure large doses of this virulent material. After a period of injection, usually
about four months, the serum of the horse is sufficiently potent to be used therapeutically. An index of high potency has been established—a high opsonic index; the ability to cause phagocytosis of meningococci in not less than 1:5000 solution of the serum; the presence of bactericidal bodies and complement-fixation bodies; the presence of antitoxic bodies and the ability to protect smaller animals against fatal doses of culture.

To recapitulate: The specific infectious agent, the meningococcus, was found to be the cause of epidemic meningitis; next the mode of infection and the pathological sequence were learned, then an immune serum was produced which experimentally, at least, was proved by biological tests to be of high potency.

The last and most important step was to apply the serum in human beings in treatment of the disease. At first it was used like other sera and antitoxins. Varying doses were injected subcutaneously and intravenously. Varying and indifferent results were obtained. Flexner first proved by experimental tests in the monkey that the antimeningitis serum injected subdurally offered best results. The subsequent clinical use of the antimeningitis serum in this way helped to definitely establish the antimeningitis serum as a reliable therapeutic agent of tremendous possibilities.

It has been found that immune sera when injected into the general circulation either by subcutaneous injection or intravenous injection are eliminated into the cerebrospinal fluid in very minute quantities. On the other hand, the injection of immune sera into the subarachnoid space is followed by very rapid elimination of the serum into the general circulation. It has been explained that epidemic meningitis once fully established is essentially a localized process, the accompanying general bacteriemia during this stage being much less important. We now see the reason for the early failure with the antimeningitis serum when injected subcutaneously and intravenously. In order to attain good results in epidemic meningitis with the specific antimeningitis serum the latter must be injected by lumbar puncture directly into the cerebrospinal subarachnoid space, where it is brought into close contact with the infected area.

It has not been proved whether or not serum injected by lumbar puncture into the subarachnoid space reaches the infected ventricles. Clinical observation certainly points to the early diffusion of such serum throughout the subarachnoid space into the ventricles. How otherwise explain the prompt clinical subsidence of symptoms, the declining of pressure phenomena, and prompt clearing up of the cerebrospinal fluid following the successful treatment with the antimeningitis serum? The little experimental work that has been done on this subject, however, does not corroborate this view. Graves, in 1912, in a series of observations on dogs, failed to demonstrate diffusion to the ventricles. Staining fluid injected by lumbar puncture could not be demonstrated in the ventricles after death. Likewise some staining fluid was added to the immune antimeningitis.
serum and injected therapeutically in a few cases. The staining material of course was innocuous. One patient died of the disease, and post mortem failed to demonstrate the staining material in the lining of the ventricles.

The last step in the elaboration of a specific, scientific treatment for this disease was the establishment of safe and correct methods of administering the antimeningitis serum. After learning that the antimeningitis serum acted locally by bathing the infected parts, it was of course thought desirable to inject as much of the specific serum as could be done safely. It was at first thought that after lumbar puncture was performed, and cerebrospinal fluid removed in any quantity, a serum equal in quantity to the cerebrospinal fluid removed could be safely injected. On this basis the dose of the antimeningitis serum was an arbitrary quantitative one, depending upon the quantity of the cerebrospinal fluid removed, believing that at least an equal quantity of fluid could be safely injected. This method in general was followed by fairly good results. The writer (15), however, in a careful study of a great many cases noticed occasionally attacks of collapse, respiratory embarrassment, convulsions, and even death, following the injection of the antimeningitis serum, the dose being determined as already explained. Believing that possibly the arbitrary method of determining the dose was unsafe, he undertook more careful study, and ultimately found, as he had at first suspected, that the arbitrary quantitative method of determining the dose was not only unsafe, but at times very dangerous and occasionally even resulted in death.

Observations on the cerebrospinal fluid pressure were first made. Readings at the beginning of puncture, during the removal of the cerebrospinal fluid and during the injection of the antimeningitis serum, were made. It was thought that if, after removal of the cerebrospinal fluid, the dose of serum were guided by the cerebrospinal fluid pressure, so that when the cerebrospinal fluid pressure became equal to that at the beginning of removal of cerebrospinal fluid, it should be considered that a full dose of serum had been administered; in this way the pressure conditions in the subarachnoid space would be reëstablished and the dangerous symptoms eliminated. It was found very early, however, that these observations were very misleading and dangerous and were absolutely no criterion as to the quantity of serum that could be safely injected.

Blood-pressure studies were then begun. The writer very soon came upon some very interesting data. He found first that the injection of the antimeningitis serum in quantity equivalent to the fluid removed did not reëstablish conditions. Removal of cerebrospinal fluid was usually accompanied by a moderate fall in blood pressure. Quite often, however, no change in blood pressure followed; other times the blood pressure rose. Injection of antimeningitis serum, however, uniformly in the great majority of cases produced a fall in blood pressure. The blood pressure dropped and continued to drop as larger quantities of fluid were injected.
The fall in blood pressure likewise depended very largely on the rate and pressure used in the injection of the serum. There was absolutely no relationship between changes in blood pressure following the removal of cerebrospinal fluid and the changes following the injection of the serum. If the injection of the serum were continued in spite of the warning fall in blood pressure, symptoms of respiratory embarrassment, shock, convulsions, and even death ensued.

As a result of these findings the writer concluded (1) that the arbitrary quantitative method of determining the dose of the antimeningitis serum was inaccurate and dangerous; and (2) that the blood-pressure changes noted during the injection of the antimeningitis serum offered a valuable guide as to the quantity of serum that could be safely injected.

**Classical Method of Performing Lumbar Puncture and Administering Antimeningitis Serum**

*Technique of Performing Lumbar Puncture*

**Anesthesia.**—General anesthesia is dangerous, and should not be employed except where positively indicated in violent, delirious patients or in highly sensitive, nervous patients. Local anesthesia is worthless. The severest pain during lumbar puncture occurs when the spinal membranes are perforated. A quick puncture, skillfully performed, is very often a mild operation.

**Site of Operation.**—The site of operation should be sterilized and draped off as for a major operation. It is desirable to select a level for puncture below the conus medullaris. In this way danger of injuring the cord is eliminated and there is less likelihood of injuring the nerve roots. The level of the conus varies in different people. In young children it is very often slightly lower than in adults. The fourth lumbar space, which is at the level of the crest of the ilium, is below the level of the conus. This site, or the lumbosacral space, is, therefore, usually selected. After a number of punctures on different days it may be desirable to select another level. Adhesions may form and shut off the subarachnoid space at the operated level, and there may be danger of infection from the irritated and inflamed skin over the puncture. The next space above, the third lumbar space, should then be chosen and, if necessary, even the second or first lumbar space may be selected. Puncture at the latter two levels, however, is attended by greater danger of perforating the cord and consequently injuring some of the important nerve centers, notably those of the bladder, rectum, or roots of the lower extremities. This danger, however, is not so imminent, since in epidemic meningitis the subarachnoid space is markedly
distended by the cerebrospinal fluid, with consequent separation of the enclosed tissues. Furthermore, at the lower level, as a rule, the posterior subarachnoid space is intact, so that the needle first taps the distended sac and there is less danger of perforating the cord.

**Posture of the Patient.**—The patient should lie well on his side over the edge of the bed. A right-handed operator should have the patient on his left side and vice versa for a left-handed operator. This will allow the right hand to be freely used. The back should be well bowed. The head should be bent as much as possible on the chest. The legs should be flexed on the thighs and the thighs on the abdomen.

Lumbar puncture should never be performed in the erect posture in cases of meningitis. It is extremely dangerous and may be accompanied by collapse and even death.

**Selection of Proper Needle.**—A large, strong, and pliable needle with large bore should be used. A good steel or, preferably, iridoplatinum needle 4 to 4 1/2 inches in length and 1 1/2 to 2 mm. in diameter will give good results. Most operators prefer a needle with a trochar, as this adds strength to the needle and enables one to clear the lumen of the needle should it be plugged by tissue or fibrin. The edge of the needle should be sharp-cutting, so that it will readily penetrate the tissues, but short-beveled, so that it will have the advantage of a blunt needle in pushing the nerve roots aside as they are met. The short bevel furthermore eliminates the danger of peridural spilling of the cerebrospinal fluid when there is only partial entry of the edge into the spinal theca.

**Method and Route of Puncture.**—The least complicated and most direct way is the median route. A very satisfactory method is as follows: Select the proper level for the operation, then place the thumb of the left hand firmly in the intervertebral space, pressing it well between the spines and holding it there as a guide for the needle, which is directed at an angle of 45° or less upward and inward between the spines. The needle should be directed rather closer to the upper border of the lower spinal process, in this way avoiding the tubercles which project downward from the lower margin of the lumbar spinal processes. As the membranes are punctured the patient frequently screams and complains of very severe burning, often shooting pains in the back around the abdomen, sometimes in the hip and down the legs. In a moment this pain disappears, but a dull, boring pain at the site of the puncture persists.

The lateral route of puncture has been advocated by some authors, principally on account of the fact that by this route the thick interspinous ligament can be avoided. A blunt needle can thus be used, and there is less danger of injuring the cord and the spinal nerve roots. This route of puncture, however, requires so much more skill and even in the hands of a
practiced operator is apt to be so much more painful that, as a rule, it is far less desirable than the median route.

**Accidents During Lumbar Puncture**

**Hemorrhage.**—Hemorrhage during lumbar puncture may result either from injury of the epidural veins, which usually occasions the flow of a rather large stream of pure blood through the needle, or from injury of the subdural veins, which, as a rule, simply causes blood tingeing of the cerebrospinal fluid. In the latter condition the cerebrospinal fluid usually clears up after a few moments, but in the former the needle, which has not penetrated the subarachnoid space, should be removed and reinserted, taking care, of course, first to remove the clot from the lumen of the needle. Neither form of hemorrhage, as a rule, is of any consequence.

**Accidental Breakage of Needle.**—This accident should never happen if a proper needle be selected for the puncture. This needle should be large and powerful. The author has seen a number of instances where the needle snapped off in the middle during an operation after the canal had been reached, caused by a sudden contraction of the muscles of the back. In almost every instance the physician had dissected extensively for the needle, but failed to find it. In none of these cases did the writer attempt to locate the needle. Several patients recovered completely and complained of no symptoms that could be explained by the presence of the needle. The author believes it most advisable in such cases to wait and ascertain how much damage is actually done before instituting radical measures. The dissection of the membrane is an extensive and difficult operation, and should not be attempted unless absolutely indicated.

**Specific Treatment of Epidemic Meningitis**

The specific treatment of epidemic meningitis varies somewhat in the acute and chronic forms of the disease.

The broad general principle in the serum therapy of acute meningitis is to inject the serum as early as possible after the beginning of the disease, always giving the patient the benefit of the doubt; in treatment always favoring the diagnosis of epidemic meningitis. The serum should be injected at the first puncture. Later the diagnosis may be corroborated by the examination of the cerebrospinal fluid.

Active subdural treatment should be kept up as long as any active signs of the infection are present, either as indicated by clinical signs or by the examination of the cerebrospinal fluid.

The hydrocephalus should be treated at the same time as the specific serum treatment is being administered.

The same attention must be paid to the general measures as in treating any acute infectious disease.
Method and Technique of Administering the Antimeningitis Serum

It has been explained that early diagnosis in treatment is most important. Lumbar puncture should be performed early if only on strong clinical suspicion of meningitis. If the fluid be increased in quantity and slightly opalescent, the serum should be injected. The usual finding in a frank case of meningitis is a large quantity of turbid fluid under considerable pressure. The absolute confirmation of the diagnosis in any case is only made later by bacteriological examination.

After performing lumbar puncture, using the precautions already explained, as much cerebrospinal fluid should be allowed to escape as can be done safely. This is controlled by the condition of the patient, his color, respiration, and pulse, and principally by the coincident observation of the blood pressure during the operation. As a rule, the cerebrospinal fluid can be allowed to escape slowly until the cerebrospinal fluid pressure comes down to normal, as actually measured by a manomètre or roughly gauged by the flow of the fluid, the normal fluid averaging about one drop every 3 to 5 seconds. Usually the withdrawal of cerebrospinal fluid is a perfectly safe procedure. The clinical condition, as a rule, is good, and the blood-pressure change is ordinarily insignificant. Most often there is a moderate fall in blood pressure, varying between 2 and 3 and 10 mm. of mercury. The writer has found by experience that a fall of 10 mm. of mercury in the blood pressure may be considered a safe guide to discontinue the further withdrawal of fluid. Sometimes the blood pressure does not change at all during the operation; at other times it may rise.

While the cerebrospinal fluid is being withdrawn the serum should be prepared by warming to body temperature.

The serum is injected through the lumen of the needle under pressure. Two general methods are used: (1) the syringe method, (2) the gravity method.

The syringe method consists simply in the injection of the serum by means of a syringe which is attached to the needle. Most of the lumbar puncture needles manufactured are made to have a standard size handle, so that the tip of the average syringe fits well into the needle.

In the other method serum is injected by gravity. The most simple apparatus used consists of the barrel of a 15 to 25 c. c. syringe used as a funnel attached to a 12 to 14-inch rubber tube about one-quarter of an inch in diameter, at the end of which is a small metal end piece or adapter, which should fit the hilt of the needle. The latter is made by most instrument manufacturers.

The serum is poured into the funnel and made to displace the air in the rubber tube. When the serum appears at the end of the rubber tube it is attached to the needle.

A number of manufacturers have placed on the market a special grav-
ity apparatus, which is fully assembled with the serum in the container and ready for use. The advantage in this is that there is no need of assembling the parts and that there is little exposure of the serum to the air.

It has been explained that the dose of serum is a variable one, and must be carefully controlled in each individual case and at each separate injection. The quantitative method of determining the dose as guided by the quantity of cerebrospinal fluid removed is dangerous, and should not be employed. It has been noted that the blood pressure falls during the injection of the antimeningitis serum and that the degree of fall may be used as a guide to the quantity of serum that can be safely injected. The writer has been accustomed to have the blood pressure reported by a special assistant throughout the whole operation, both during removal of fluid and during injection of serum. As a result of observations in many cases, he has found that a total fall of 20 mm. of mercury in a person with an initial blood pressure of 110 to 120 mm. of mercury indicates that the further injection of serum should be stopped. The same holds true in young people with a high blood pressure, since the latter in meningitis is most often a direct result of the hydrocephalus, so that patients with an initial blood pressure of 160 mm. of mercury, or even higher, also cannot usually bear more than a fall of about 20 mm. A slightly greater relative fall in blood pressure may be allowed in children. The degree of fall in blood pressure that may be safely allowed during the injection of serum can be fairly well determined by considering a fall of 20 mm. safe for a blood pressure of 110 mm. of mercury or over for an adult; and for children the same relative fall may be allowed.

As a rule, the blood pressure begins to fall shortly after the injection of the antimeningitis serum has been begun. The amount of fall is dependent upon the quantity of fluid injected and the rate and pressure of the injection. The writer has found clinically, and Dr. Carter has confirmed by experimental observations in dogs, using Ringer's solution for intraspinal injection, that the latter two factors of rapidity and pressure of injection are most important; that a small quantity of fluid injected rapidly under considerable pressure will cause relatively much greater fall in blood pressure than a large quantity of fluid injected slowly and with little pressure. The great advantage of the gravity method is that the rate and pressure can be much more accurately controlled simply by raising or lowering the funnel holding the serum. The disadvantage of the syringe, furthermore, is that the piston may "stick," and, in exerting force to push it on, a little serum may be suddenly injected under very considerable pressure.

The fall in blood pressure is usually gradual and progressive up to a certain point, usually to about 20 mm. of mercury in an adult. Beyond this point, if the injection of serum be further continued, the blood pressure may fall very suddenly and be accompanied by the very severe clini-
cal symptoms of shock, collapse, and even death. Thus 30 c. c. of serum, for example, may be injected into an adult accompanied by a fall of 20 mm. of mercury. If a few more cubic centimeters of serum be injected a very large fall may occur. The author has seen a sudden fall of 100 mm. of mercury in robust subjects when only 4 c. c. of serum were injected after the initial fall of 20 mm. of mercury in blood pressure.

If the blood pressure has fallen to a dangerous point before an adequate dose of serum has been injected, one should wait a few minutes. Not infrequently the blood pressure will rise a bit, and then a little more serum can be injected. If the blood pressure does not change after the first fall, one may proceed very cautiously. If, on the other hand, the blood pressure continues to fall, even after the injection of serum has been stopped, under no circumstances should more serum be administered.

Fifteen to twenty minutes may be considered a safe interval of time to allow for the injection of a full dose of serum.

The average dose of serum when controlled by blood pressure is as follows:

<table>
<thead>
<tr>
<th>Age Range</th>
<th>Amount of Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 to 5 years</td>
<td>3 to 12 c. c.</td>
</tr>
<tr>
<td>5 to 10 years</td>
<td>5 to 15 c. c.</td>
</tr>
<tr>
<td>10 to 15 years</td>
<td>10 to 20 c. c.</td>
</tr>
<tr>
<td>15 to 20 years</td>
<td>15 to 30 c. c.</td>
</tr>
<tr>
<td>20 years and over</td>
<td>20 to 40 c. c.</td>
</tr>
</tbody>
</table>

(Occasionally more)

These doses, though in many instances smaller than formerly used, give very much better results than the larger doses injected without adequate control.

The clinical symptoms accompanying the fall in blood pressure during the injection of the antimeningitis serum consist principally of deep stupor, severe respiratory embarrassment, and general symptoms of severe shock. The breathing first becomes irregular, slow, stertorous; sometimes very superficial, rapid, and irregular. The color becomes livid; other times, cyanotic. The pupils dilate and there is incontinence of feces and urine. The pulse quite often remains good at first, but later grows weak, rapid, and irregular; other times, slow and irregular.

With the appearance of symptoms active treatment should at once be instituted. The head of the patient should be raised and as much of the injected fluid removed as necessary. In using the gravity method this can be easily done by simply lowering the container holding the serum. If the blood pressure rise and the general condition of the patient improve following these measures, a little of the fluid can be reinjected. If the breathing be poor or stertorous, artificial respiration should be actively administered. Atropin in doses of 1/80 to 1/50 of a grain and eocain in doses of 1/8 to 1/4 gr. should be injected hypodermatically. Atropin relieves the cardiac inhibition and eocain relieves the respiratory embarrassment.
Carter (5), in his experimental studies on the intraspinal injection of Ringer’s solution in dogs, found that the first mechanical effects of the increase in the intraspinal pressure were respiratory embarrassment and marked cardiac inhibition.

**Case I.** Man, aged 22, came under treatment on the second day of his illness. He was severely ill with acute epidemic cerebrospinal meningitis. Blood pressure was 130; general condition good. Lumbar puncture yielded a very turbid fluid under high pressure. Fifty c.c. of fluid were removed, accompanied by a fall of 5 mm. of mercury in blood pressure. When the cerebrospinal fluid pressure dropped to normal, further withdrawal of fluid was discontinued. Antimeningitis serum which had been warmed to body temperature was then injected by gravity. The injection of the first 10 c.c. of serum caused no change in blood pressure. The further injection of serum, however, occasioned a gradual fall in blood pressure as the larger quantities of serum were injected. When 25 c.c. of serum had been injected, the total fall in blood pressure had been 18 mm. of mercury. The injection of serum was then stopped and the blood pressure carefully watched. The fall in blood pressure, however, continued to a total drop of 22 mm. of mercury. After waiting two minutes and there being no tendency for the blood pressure to rise, it was decided that a safe dose had been administered and the needle was removed from the spine. The patient’s clinical condition was good and he left the table very little the worse for the operation. Eighteen hours later the patient’s general condition was very much improved. His blood pressure at this time was 115. Lumbar puncture yielded a very turbid fluid under very high pressure. The removal of 35 c.c. of fluid caused a drop of 10 mm. The further withdrawal of fluid was therefore stopped and the injection of serum was begun. After 15 c.c. of serum were injected by the gravity method the total fall in blood pressure was 20 mm. of mercury. The blood pressure, too, exhibited a tendency to continue falling in spite of the fact that injection of serum was stopped for the moment. It was decided, therefore, to discontinue the further injection of serum. The patient left the table in good condition.

Steady improvement continued and temperature became normal. Twenty-four hours later another puncture was performed. The blood pressure at this time was again 135. Lumbar puncture yielded almost a clear fluid under considerable pressure. Fifty c.c. were removed, accompanied by a fall of 5 mm. of mercury. The further withdrawal of fluid was discontinued when the cerebrospinal fluid pressure fell to normal. Serum was injected by the gravity method. Twenty c.c. were injected and blood pressure fell only 5 mm. It was considered, however, that a dose of 20 c.c. was sufficient in view of the marked clinical improvement and the clearing up of the cerebrospinal fluid. The patient left the table in excellent condition, perfectly conscious and feeling well. From this time on he progressed to an uninterrupted recovery, without further treatment.

**Case II.** Mulatto, aged 25, admitted to the hospital on the sixth day of his illness, violently ill, delirious and severely prostrated. Blood pressure was 70. Lumbar puncture yielded a thick, purulent fluid. Twenty c.c. were slowly removed without any change in blood pressure. The injection of serum by the gravity method was almost immediately followed by increasing fall in blood pressure. The injection of 10 c.c. of serum caused a fall of 15 mm. of mercury in blood pressure. Thirteen c.c. of serum were followed by a fall of 20 mm. The injection of serum was discontinued. The needle, however, was left in situ for a few minutes while the blood pressure observations were carefully made. In spite of the discontinuation of serum injection the blood pressure continued to fall,
TREATMENT OF EPIDEMIC MENINGITIS

dropping in all 30 mm. of mercury. At this point the patient's general condition became very bad. His color became pasty; breathing very superficial and irregular. He began to have incontinence of stool. At once the head of the patient was raised and serum was removed from the subarachnoid space. Upon the removal of 8 c.c. of serum the blood pressure commenced to rise, recovering 10 mm. of mercury; with the rise there was coincident improvement in the general condition of the patient. He was watched for about half an hour, the needle all this time being left in situ. Improvement, however, was steady and he left the table in good condition.

Twelve hours later there was but little improvement in the patient's condition. He was totally unconscious and his general condition was poor. Blood pressure was 80. Another lumbar puncture was performed. The cerebrospinal fluid was considerably thinner, under very appreciable pressure and still very purulent. Sixty c.c. were removed, accompanied by a rise to 100 mm. of mercury in blood pressure. Injection of serum was, nevertheless, again immediately accompanied by a rapid fall in blood pressure. Injection of 20 c.c. of serum occasioned a total fall of 25 mm. of mercury. (This included the gain which occurred during the removal of fluid.) Again the patient's condition became bad. Upon the withdrawal of 5 c.c. of serum there was immediate improvement.

This patient made a recovery after eight injections of serum. At no time was he able to bear more than 20 c.c. of serum without the development of alarming symptoms and very pronounced fall in blood pressure.

Case III. Woman, aged 35, admitted to the hospital after an illness of three days. She was totally unconscious and violently ill. Her blood pressure was 100 mm. Lumbar puncture yielded a moderately turbid fluid under considerable pressure. Eighty c.c. were removed, accompanied by a fall of 5 mm. of mercury. Serum was then injected by the gravity method. Ten c.c. were injected with no change in blood pressure. The injection of larger quantities of serum, however, was immediately followed by a steady and progressive fall in blood pressure; 15 c.c. of serum caused a total fall of 15 mm. of mercury; 18 c.c. of serum a fall of 20 mm. of mercury. In view of the patient's serious condition it was thought desirable to attempt to inject a somewhat larger dose of serum. Twenty-five c.c. of serum were accompanied by a fall of 25 mm. of mercury; 28 c.c. of serum by a fall of 60 mm. of mercury. At this point the patient suddenly stopped breathing. Her head was promptly raised; serum, 12 c.c. in all, was removed rapidly from the subarachnoid space; artificial respiration was instituted; 1/6 of a grain of cocaine was administered hypodermatically. After a few moments the patient began to breathe; heart action again became good. She left the table in fair condition, though, undoubtedly, the severe shock had left its mark. Fourteen hours later, her condition was worse; blood pressure was 105 mm. Lumbar puncture yielded a fluid very much the same as the first. Fifty c.c. were removed with a fall of 10 mm. of mercury. The injection of 15 c.c. of serum occasioned a total fall of 20 mm. of mercury. It was decided to discontinue for the moment the further injection of serum, but to leave the needle in situ and to watch the blood pressure carefully. After waiting five minutes there was no further drop in blood pressure. Five minutes later the patient recovered 10 mm. of the fall, making the total fall only 10 mm. of mercury. It was decided to continue the injection of serum and 10 more c.c. of serum were administered, again causing a fall of 10 mm. of mercury. After watching the patient for a few minutes to make certain that there would be no subsequent fall in blood pressure the needle was removed, the patient leaving the table in good condition. This patient died after being treated for three more days.
Observations on Concentrated Antimeningitis Serum.—The principle applied in the refinement and concentration of immune sera consists in the elimination of the albumin and euglobulin from the serum, leaving only the pseudoglobulin, with which protein the immune bodies are closely associated. The method now generally employed is that devised by Gibson (8) in the New York Research Laboratory, subsequently modified and improved by Banzhaf.

While in the Research Laboratory the writer had several liters of antimeningitis serum concentrated, and made some observations on this refined serum in the subdural treatment of epidemic meningitis. Believing that many of the ill effects occurring during the injection could be explained by the quantity of fluid injected, he thought that by reducing the quantity of serum injected, without diminishing the number of immune bodies, he might obtain better results. The serum was concentrated to one-third the original volume. About 12 cases in all were treated at different times with this serum. The dose of serum was one-third to one-half less than the dose of the usual unrefined serum, but the actual number of immune bodies injected was relatively greater.

The results, however, were disappointing; very little, if any, advantage over the unrefined serum was noted, even though full doses were used in a few instances. This may well be explained. The principal virtue of the antimeningitis serum is the production of a local leukocytosis and phagocytosis; this is accomplished most thoroughly when the serum bathes the infected parts freely; a small quantity of serum, though relatively more potent, could not come in as close contact or freely bathe as large a surface and so failed to give as good results as the less potent serum.

Further observations on this subject should be made.

Effect of Preservatives in Serum.—Another feature of the antimeningitis serum might be cited here. All immune sera used therapeutically are rendered sterile and bacteria-free by passage through a Berkefeld filter. Preservatives, such as chloroform and tricresol, are also usually added. In the case of antimeningitis serum, particularly, the use of a preservative is desirable, since a serum accidentally injected into the meninges would gravely jeopardize the life of the patient. The Bureau of Hygiene, supervising the interstate sale of biologic products, has permitted the use of .4 per cent. tricresol, which has been used in most instances. The writer at different periods has worked with serum without and with tricresol, and in an attempt to explain some of the severe pain, restlessness, and discomfort which sometimes follow the injection of the antimeningitis serum made some observations on the effect of serum with different strengths of tricresol when injected into the brain of the rabbit. Serum with .4 per cent. tricresol made the animal very restless, and sometimes caused convulsive seizures and retraction of the head. Serum with .2 per cent. or less tricresol did not produce these symptoms.
TREATMENT OF EPIDEMIC MENINGITIS

The writer believes that the large quantities of tricresol permitted as a preservative in the antimeningitis serum are temporarily irritating, though he does not believe, as suggested by some, that this quantity of preservative in antimeningitis serum is very dangerous or has led to death. Sera with .4 per cent. tricresol injected directly into the ventricles of the brain, in treating posterior basic meningitis, have been as well borne, without ill effects, as when injected by lumbar puncture. A smaller quantity of preservative should, however, be used.

The beneficial effect of the injected serum is indicated very often 8 to 24 hours after the injection. The temperature may rise for a few hours after the operation, but with favorable response falls later. Quite frequently it becomes normal 24 hours after the first dose of serum. The most striking evidence of improvement is in the rapid clearing up of the cerebral symptoms, the disappearance of delirium, and the relapse into a quiet, restful sleep. There is often a prompt improvement in Kernig's sign, the rigidity of the neck, and the other evidences of active meningeal inflammation.

The most important sign of improvement, however, is the clearing up of the cerebrospinal fluid. Before treatment is begun, or if there be no response to serum treatment, the fluid is usually turbid under high pressure, and shows microscopically many pus cells, meningococci, most of the coci being extracellular and relatively few intracellular. One of the most important functions of the antimeningitis serum is to stimulate phagocytosis. Response to a dose of serum, therefore, is best indicated by the diminution in the total number of meningococci and by the inclusion of the meningococci within the leukocytes; with no improvement there is an increase in the number of meningococci, and most of the organisms are extracellular.

The indications for repeating the doses of serum are the change in the clinical condition of the patient under treatment and the change in the cerebrospinal fluid. Treatment should be actively kept up until either all meningococci have disappeared from the cerebrospinal fluid or until there are only a few meningococci and those all intracellular. Even few extracellular meningococci signify that the dose of serum should be repeated the following day, although the clinical condition of the patient continues good.

If the cerebrospinal fluid show no meningococci, and the clinical condition of the patient be unsatisfactory, then a dose of serum should likewise be repeated, since it is evident that the infection is still present and that most probably a few extracellular meningococci have been overlooked in the examination.

If the clinical condition of the patient be good, and if the previous fluid had shown few meningococci and those intracellular, then it is perfectly
safe to omit the dose of serum that day, repeating it only as is subsequently indicated by the course of the disease.

The average case requires daily injections for 3 to 4 days. Severer cases may require a few more doses. After the first three or four doses of serum it is desirable to allow a longer interval between the subsequent doses. Injections on alternate days or even less often, as controlled by the condition of the patient, have the advantage of giving the patient a period of time during which he may not only respond to the previous dose of serum, but also recuperate from the shock of the injection itself. The system, too, is so well saturated with the serum after a few doses that the daily doses are not urgently indicated. Some cases require treatment for a long time—as many as twenty or more doses being necessary.

The intraspinal serum treatment of cases with thick, plastic exudate is difficult and often dangerous. The cerebrospinal fluid is viscid, contains large clumps of fibrin, and is too thick to flow through the lumen of the needle. Injection of the antimeningitis serum under pressure without previously removing the cerebrospinal fluid is very dangerous. One should first attempt to start the flow of the cerebrospinal fluid by gently irrigating with a little sterile salt solution injected through the needle under a little pressure. If this fail two needles may be introduced into the subarachnoid space at different levels so that the solution may be injected at one space and come out at the other. If the latter method prove ineffectual also, one should then administer the serum under pressure, taking great care to inject only a small quantity at one sitting and to note carefully the effect on the blood pressure during the injection. The treatment may be repeated at more frequent intervals than in the average case. A few doses may be administered at 8-hour intervals. This method of treatment is successful in a fair proportion of cases. After one or two doses of serum the cerebrospinal fluid quite often becomes less viscid and flows well through the lumen of the needle.

*Case IV. Thick Plastic Exudate. Boy, aged 10, admitted to the hospital on the sixth day of his illness. He was violently delirious and had all of the pronounced clinical symptoms of the most virulent form of epidemic meningitis. Blood pressure, 90. Lumbar puncture yielded a few cubic centimeters of very thick, viscid, creamy cerebrospinal fluid. A few strings of fibrin occluded the lumen of the needle. This was removed with the trochar, but after the escape of a few more drops of fluid the lumen was again clogged. It was then thought advisable to gently irrigate with warm saline solution. Two to three c.c. of saline were injected and allowed to escape promptly. This was attended by but poor results. Serum was then injected under pressure. Six c.c. of serum were immediately followed by a fall of 15 mm. of mercury. The patient became more stuporous, and his breathing became shallow and irregular. Further injection of serum was then discontinued. Eight hours later there was little change in his condition. It was then decided to again puncture. The blood pressure was now 70. Lumbar puncture again yielded a few cubic centimeters of thick, viscid fluid. Another needle was now introduced into the next lumbar space above. Warm
saline was injected in the upper needle and allowed to drain out in the lower. At first, there was no response, but after the introduction of a few cubic centimeters of fluid in this way the flow of cerebrospinal fluid became much freer. In all 15 c.c. of fluid were removed. There was no change in blood pressure. Serum was then injected. This time a total of 12 c.c. of serum was injected before there was a fall of 15 mm. of mercury with severe symptoms of shock. Injection was then stopped. Twelve hours later the patient's condition had improved considerably. He was more conscious, his general condition was better. Blood pressure now was 110. Lumbar puncture yielded a very turbid fluid which flowed readily. Forty c. c. were removed, with a fall of 10 mm. of mercury. Twenty-five c. c. of serum were injected before there was a fall of 20 mm. of mercury, when the further injection of serum was discontinued. This patient ultimately recovered.

A similar problem is faced in treating cases with dry canal, giving a so-called dry puncture. Most often the so-called dry puncture really means failure on the part of the operator to enter the subarachnoid space. True dry puncture, however, does occur. It is not infrequently encountered during serum treatment. When accompanied by coincident evidence of clinical improvement the condition may be interpreted favorably and serum treatment omitted at that sitting. Sometimes, however, grave signs of local and general sepsis are seen with true dry puncture. In these cases persistence of the infection, possibly in localized and encapsulated areas throughout the subarachnoid space and within the ventricles, is very probable. Treatment should be continued and serum injected under pressure. Cases like those with thick, plastic exudate often clear up under this mode of serum treatment. The third very important form of dry canal is that present in posterior basic meningitis, in which the subarachnoid space is dry and, through closure of the basal foramina, shut off from its communication with the ventricles of the brain. The latter, in turn, usually contain a large quantity of exudate under considerable pressure. Intraspinal serum treatment of this condition is not only useless, but very dangerous, since the focus of infection, located within the ventricles of the brain, is not reached by the injection into the subarachnoid space; the fluid so administered under pressure would cause grave pressure symptoms. The special treatment for this condition will be described later.

Serum Treatment of General Bacteriemia Immediately Preceding and During the Course of Meningitis

It will be explained further on that meningitis begins as a local nasopharyngitis, which in a certain percentage of cases is followed by general bacteriemia. The latter lasts on the average between 8 and 36 hours, and may terminate in one of several ways. It may terminate in recovery, as seen in the so-called aborted cases during an epidemic of meningitis. It
may result in death, as in the cases of terrific general sepsis, often accom-
panied by very profuse petechial and purpuric eruptions, which show slight
or no signs of meningitis, but which terminate in death very shortly after
the onset of the disease. These are the true fulminating cases. Most often,
however, the general bacteriemia after a certain number of hours is suc-
ceded by localization of the infection in the meninges, followed by the
classical infection of epidemic meningitis. The general bacteriemia in
these cases dies out to a very great extent a short time after the onset of
the meningitis proper. A moderate bacteriemia persists, however, in a
fair proportion of cases.

The first condition to be met, therefore, is the premeningitic stage.
The rapid fatal outcome of the fulminating cases may be prevented in
some instances, and the average cases which run the usual course of
meningitis may be considerably improved, so that when meningitis
proper sets in the infection will be much milder and to a degree under
control.

Correct, accurate diagnosis during this important period of premening-
gitis is so very difficult that, unfortunately, this stage is often overlooked.
During epidemics of meningitis, however, physicians should be on the look-
out for the disease. Careful weighing of symptoms during an epidemic
will, in some cases, permit a tentative diagnosis.

The principal symptoms of this stage may be grouped under two head-
ings: (1) general sepsis, with history of exposure; (2) hydrocephalus.
The symptoms of general sepsis, evidenced by the chill, fever, and pro-
stration, are very much the same here as in other forms of general sepsis.
Most significant manifestations, however, are severe general petechial
eruptions or purpura, crops of herpes on the face, conjunctivitis, together
with the laboratory findings. Blood examination demonstrates a moderate
leukocytosis with high relative polynucleosis. Examination of the secre-
tion of the herpes quite often exhibits a few meningococci (Gram-negative
diplococci), and examination of the urine will in a small percentage of
cases demonstrate large numbers of Gram-negative diplococci. Blood cul-
ture, while very often positive during this stage, is, of course, of no value
for rapid early diagnosis.

The early presence of hydrocephalic symptoms can be explained by the
special affinity of the meningococcus for the meninges. This affinity of the
toxic products during the stage of general bacteriemia probably accounts
for the early irritation and collection of clear fluid within the ventricles
and subarachnoid space and the subsequent localization of the meningococ-
cus in the meninges with the onset of the true meningitis. The significant
clinical symptoms due to this condition are the violent, persistent headache,
which cannot be explained by the usual causes; the early, repeated, explo-
sive vomiting, which is not accompanied by evidence of any gastro-intes-
tinal disorder and cannot be controlled by local treatment; the early hyper-
esthesia, irritability, and photophobia; the dilated, sluggishly responding pupils, the tenderness at the angles of the jaws and the presence of the bulging fontanel in young children or the Macewen sign in the older, and, most important, the irregular pulse and respiration. Treatment of this condition, even on suspicion, should consist of lumbar puncture with removal of a large quantity of exudate followed by the injection of a small dose of serum into the subarachnoid space. A larger dose of serum up to 100 c. c. should at the same time be injected subcutaneously or intravenously. The general bacteriemia will, in a measure, be controlled by the injection of the serum into the general circulation, and the hydrocephalus will be relieved by the removal of fluid. The injection of a small dose of serum into the subarachnoid space also helps to take care of any infection which may already be localized in the meninges.

Case V. Girl, aged 19, was seen by the writer 18 hours after the beginning of symptoms. She did not appear very ill and had only slight fever. She complained of persistent headache, had occasional attacks of projectile vomiting, felt dizzy, was irritable and restless, and complained of pain at the nape of the neck. The pupils were widely dilated and responded very sluggishly to light. She had a crop of herpes on the upper lip and had a few petechial spots over the extremities. These symptoms of moderate hydrocephalus and mild sepsis, occurring during an epidemic of meningitis, warranted a tentative diagnosis of the first or premeningitic stage of meningitis. Many of the active signs of meningitis were missing. Neck rigidity was absent, as was also the Kernig sign and many of the other classical signs of epidemic meningitis. Lumbar puncture yielded a clear fluid under very high pressure, 45 c. c. of fluid being removed. Fifteen c. c. of serum were injected intraspinaly. At the same time 30 c. c. of serum were injected underneath the skin. An examination of the cerebrospinal fluid showed a slight increase in protein content, 50 cells per c. mm., most of the cells being lymphocytes, and the examination of the smear showed a few Gram-negative extracellular diplococci, which, however, failed to grow in culture. The diagnosis was apparent. The disease was either in the premeningitic stage (stage of general bacteriemia) or just at the very beginning of the meningitic stage. The presence of a few extracellular organisms in the cerebrospinal fluid, however, did not necessarily mean that the bacteria had already localized in the meninges, since these bacteria could be explained by the coincident general bacteriemia.

This patient made a prompt, uninterrupted recovery and was discharged as well 4 days later without any further treatment. The prompt recovery here could be explained by the treatment of the general bacteriemia through the subcutaneous injection of serum, the treatment of the possible beginning of the local infection in the meninges by the serum injected into the subarachnoid space and the relief of hydrocephalus with the removal of cerebrospinal fluid.

Case VI. Woman, aged 45, admitted to the hospital in a state of complete collapse after an illness of eight hours. She was cyanotic and almost pulseless. She was perfectly conscious, however, and complained only of a severe headache, vertigo and vomiting. Her neck was limber and Kernig’s sign was absent, but the pupils were widely dilated and Macewen’s sign was present. She was also exquisitely tender upon pressure at the angles of the jaws. Her body was covered with a very profuse petechial eruption. Temperature was subnormal. An examination of the urine showed many pus cells and many extra- and intracellular
Gram-negative diplococci. (A history of gonorrhea could be absolutely excluded.) Blood examination showed white blood corpuscles, 12,000; polymorphonuclears, 80 per cent.

This case was evidently a severe, fulminating type of epidemic meningitis. The terrific onset, prostration, with the profuse petechial eruption, accompanied by the presence of Gram-negative diplococci in the urine, indicated a severe general bacteriemia. The symptoms of headache, vomiting, dilated pupils, and the Macewen sign indicated moderate hydrocephalus. Lumbar puncture was performed, and 20 c. c. of absolutely clear fluid under moderate pressure were removed. In view of the severe prostration, it was thought inadvisable to inject serum. Sixty c. c. of serum were injected subcutaneously; active stimulation for shock was also promptly applied. For a period of 18 hours the patient needed constant attention; two intravenous infusions of saline solutions were necessary, with other very active stimulation. Her general condition then suddenly improved; color became better, heart action much stronger. Temperature, however, now rose to 103°, and more active signs of meningitis appeared. The neck became very rigid, Kernig's sign marked, and Macewen's sign more pronounced. Lumbar puncture yielded a very turbid fluid under high pressure, 60 c. c. being removed. Thirty c. c. of serum were injected. The examination of the cerebrospinal fluid showed many diplococci, mostly intracellular. The urine, however, now failed to show any organisms whatsoever. This patient recovered after three more doses of serum, though joint involvement, which occurred on the third day of the illness, persisted for a few weeks.

Had serum been injected intraspinaly at the first lumbar puncture it is possible that the severe subsequent meningitis might, in a measure, have been prevented. The patient's general condition, however, absolutely prohibited the intraspinal injection of serum at that time. The writer is inclined to believe, furthermore, that little if any good would have resulted, since it was fairly evident that at the time, at least, the patient was suffering not from meningitis, but from a severe, overwhelming, general bacteriemia. This was treated and partly controlled by the subcutaneous injection of the serum. It would have been more desirable to inject the serum intravenously, but this was not done on account of the patient's precarious condition.

The treatment of general bacteriemia during the course of meningitis is, in a measure, controlled by the serum, which is injected subdurally, since it has been explained that serum injected into the subarachnoid space are excreted into the general circulation very quickly.

If one be unable, however, to inject suitable doses of serum into the subarachnoid space, the general bacteriemia may be coincidentally treated by injection of the serum subcutaneously and intravenously.
TREATMENT OF EPIDEMIC MENINGITIS

TREATMENT OF HYDROCEPHALUS DURING THE ACUTE STAGE OF MENINGITIS

While the condition of hydrocephalus is very important, it does not, as a rule, require any special treatment during the acute stage of meningitis, since during the usual course of serum treatment hydrocephalus is relieved at the time of each serum administration. The cerebrospinal fluid is first withdrawn before serum is injected. The severity of the hydrocephalus, too, is in proportion to the degree of the local sepsis. Thus, when a dose of serum is necessary for the local infection, coincident treatment for hydrocephalus is also indicated. Occasionally, however, very severe pressure phenomena may set in a few hours after an injection of serum. The patient may become very stuporous or totally unconscious, the breathing growing very stertorous and irregular, the heart action bad. Lumbar puncture for relief of hydrocephalus without serum injection is then indicated. Prompt relief usually follows.

Case VII. Boy, aged 19, ill three days with epidemic meningitis. There was fair response under serum treatment, but eight hours after the second dose of serum the patient suddenly developed an alarming group of symptoms. He became wildly delirious and unmanageable; his heart action became rapid and irregular; breathing became very rapid and superficial, at times slowing down with long periods of apnea. The pupils were widely dilated and slight internal strabismus developed. MacEwen's sign was very pronounced. It was evident that the patient was suffering from a sudden exacerbation of severe hydrocephalus. The temperature was lower and the previous improvement of septic symptoms pointed against sepsis as being the possible cause of these symptoms, although, of course, an aggravation of the local cerebrospinal infection would probably also be accompanied by an increase of the hydrocephalus. The occurrence of the symptoms, however, a few hours after the injection of the serum seems to indicate that the hydrocephalus might be traced to the injection of the serum proper—a condition which is occasionally seen a few hours after the injection of the antimeningitis serum. Lumbar puncture was performed and 85 c.c. of cerebrospinal fluid, moderately turbid, under very high pressure were removed. No serum was injected. The patient promptly became quiet, the delirium subsided and he fell into a quiet, deep sleep. Breathing became regular, heart action good, color excellent. He woke 8 hours later, perfectly conscious, with a normal temperature, well on the road to recovery. He required one more dose of serum 48 hours later, but after that made an uninterrupted recovery without further treatment.

A varying degree of hydrocephalus, due to the collection of a bacteria-free exudate, usually persists for a few days or longer after the infection proper has cleared up under serum treatment. Sometimes the hydrocephalus is severe and pressure symptoms distressing. Here again lumbar puncture with removal of cerebrospinal fluid gives prompt relief.

A tardy convalescence will often immediately improve after this simple measure. During the course of serum treatment, if only a few bacteria be
present, but relatively large quantities of fluid, it may be well to tap one
day and, if necessary, inject serum the next day. Relief of the local pres-
sure with improvement of the local circulation will often enable the
meninges to take care of the remaining bacteria without necessitating the
special injection of serum.

The average case of meningitis requires daily administration of serum
for 3 or 4 days. If improvement be steady, at the end of this time the
cerebrospinal fluid will often be clear but considerable in quantity. It may
be sterile or have only a few bacteria. In either instance it is often prefer-
able simply to tap the fifth day if pressure symptoms so indicate, and not
inject serum. If any septic phenomena be still present a day later a dose
of serum may then be injected.

**TREATMENT OF SUBACUTE AND CHRONIC MENINGITIS**

For purposes of study chronic meningitis may be divided into the se-
vere form, the mild form, and posterior basic meningitis.

The severe form of chronic meningitis is a continuation of a severe
acute meningitis in a chronic state. Infection is persistent and hydro-
cephalus severe. Treatment should be along the lines set forth for the
acute stage. It may be well to allow longer intervals between the doses of
serum and in the period between the doses to tap and relieve pressure.

The meningococcus vaccine, preferably autogenous, may be used. A
small dose of 50,000,000 to 100,000,000 killed meningococci should be in-
jected at first and gradually increased to the larger doses until response
is observed. Intervals between the doses of vaccine depend upon the re-
action and the response. As a rule, 3-day intervals are satisfactory.

*Case VIII.* Girl, aged 14, was seen by the writer 2½ weeks after the begin-
ning of her illness. During this period she had had two doses of serum, but
active intraspinal treatment had not been administered. She presented all of
the usual signs of meningitis with pronounced hydrocephalus. In addition she was
markedly emaciated, very stuporous and appeared to be blind. Daily lumbar
puncture with removal of cerebrospinal fluid, followed by the injection of serum,
was performed for the next 7 days. There was temporary improvement after the
first few treatments; the patient became more conscious, and appeared to see.
After a week, however, she lapsed into her former state. Treatment was now
administered every other day, then every third day. Hydrocephalus was pro-
nounced and the fluid remained persistently turbid with extra- and intracellular
meningococci in great numbers. She was evidently suffering from the severe form
of chronic epidemic meningitis. After 10 days of this treatment meningococcus
autogenous vaccine was made and treatment begun, at first with 50,000,000 killed
organisms, later with larger doses until 1,500,000,000 killed meningococci were
injected every 5 days. The patient lingered for one month and finally died.

*Case IX.* Man, aged 35, admitted to the hospital one week after his illness.
He had had one dose of serum injected intraspinally on the fourth day of his
illness with no subsequent treatment. The diagnosis was evidently that of a moderately severe case of epidemic meningitis. He was actively treated, being injected daily for 4 consecutive days with a suitable dose of antimeningitis serum. The cerebrospinal fluid cleared up markedly, though a few extracellular meningococci persisted and a moderately severe hydrocephalus continued. He was given two more doses of serum at 48-hour intervals and then apparently seemed to be well on the road to recovery. All bacteria had evidently disappeared, though a moderate hydrocephalus persisted. He continued well for 4 days, no treatment being given during this period. He then suddenly began to complain of severe headache; he vomited and his temperature shot up to 102° F. His general condition, however, was good, the neck only slightly rigid, the Kernig slight. Macawen, however, was marked. Lumbar puncture yielded an almost clear fluid under very high pressure. Sixty c.c. were removed. Twenty c.c. of serum were injected. An examination of the cerebrospinal fluid showed a few extracellular meningococci in smear but no growth in culture. After this treatment there was a prompt response and the patient continued well for one week, when once more a similar group of symptoms appeared. Again lumbar puncture was performed. This time 100 c.c. of clear cerebrospinal fluid were removed and 15 c.c. of serum later injected. The examination of the sediment demonstrated a few clumped bodies which looked very much like clumped meningococci. Culture was sterile.

We were evidently dealing, therefore, with a mild case of chronic meningitis of which the chronic hydrocephalic symptoms predominated and with it a mild, persistent infection continued. Vaccine treatment was then instituted, using an autogenous vaccine. He was injected at 3-day intervals with 100,000,000 killed meningococci. No further symptoms developed and after one month the patient was permitted to go home.

**Milder Form of Hydrocephalus.**—This form consists principally of a moderate hydrocephalus with a mild persistent infection. The hydrocephalus should be treated by repeated regular tap with simple removal of fluid daily or every other day or less often, depending upon the pressure symptoms. Occasionally tap with removal of fluid will give comfort and relief of all symptoms for a period of a week or longer; a puncture at that time will again yield similar results. It is dangerous, however, to allow the long intervals of a week between the punctures, since these cases are apt to gradually lapse into severe emaciation, increasing stupor, palsy, and finally death. Treatment should be more active and simple drainage or injections made at shorter intervals.

Sepsis should be treated by occasional injection of serum. The guides for repeating the dose are found chiefly in the change of the cerebrospinal fluid. With improvement there is a reduction in the number of meningococci, their inclusion within the cells, and finally their total disappearance. Frequent injections of serum are not as well borne in this, the chronic form of meningitis, and longer intervals of a few days must be allowed between the different doses.

Vaccine in this condition is very helpful, and will often easily take care of the slight, persistent infection. The general rules for administering
the vaccine are the same as explained for the severe form of chronic meningitis.

Posterior Basic Meningitis.—This condition consists of the shutting off of the basal foramina, through which the fluid in the subarachnoid space communicates with that in the ventricles. The infection in the ventricles becomes localized and hydrocephalus becomes extreme. The inflammation in the subarachnoid space becomes negligible so that, while at first a few cubic centimeters of infected fluid may be obtained by lumbar puncture, after a few days lumbar puncture either results in a dry tap or yields only a few drops of sterile fluid. Occasionally the condition occurs during the acute stage of meningitis; most often, however, it occurs late in the disease either during the chronic stage or during the apparent convalescence from the acute stage of meningitis. Pressure symptoms are most severe and form the striking feature of the clinical picture; septic symptoms are relatively insignificant. At first the fluid encapsulated within the ventricles is infected and contains many meningococci. This condition may persist to the very end. Most often, however, after a few days, the fluid within the ventricles becomes spontaneously sterile, though the quantity of fluid does not diminish. The rapid reaccumulation of fluid has partly been explained by the occasional thrombosis of the veins of Galen with the resulting hyperemia.

The first consideration in treatment is to recognize the futility and danger of intraspinal injection of serum. The only possible hope, slim though it may be, is by direct tapping of the ventricles. The object of treatment and the indications are the same as in the intraspinal treatment of the average case of meningitis. In the latter puncture removes cerebrospinal fluid from the subarachnoid space, and from the ventricles of the brain; the serum injected bathes the infected meninges and ventricles. In posterior basic meningitis the infection is localized in the ventricles and can only be reached by direct ventricular puncture. After ventricular puncture the indications for simple removal of cerebrospinal fluid or injection of antimeningitic serum are the same as in the intraspinal treatment of the usual acute case of meningitis.

The instruments for the operation of intraventricular puncture are the same as for lumbar puncture. One must be especially careful to keep the trochar of the needle in situ while inserting the needle through the brain tissue, since otherwise the lumen of the needle will become clogged with brain tissue.

In children with open fontanel the operation is relatively simple. The ventricles are very much dilated and the cortex thin, so that a needle introduced in almost any direction will easily enter the cavity of the ventricles. The extreme lateral border of the anterior fontanel should be selected. The needle should be directed downward, slightly backward and inward to a depth of 2 to 4 cm. or more. When the needle enters the cavity it usually
"gives." The head should be turned to the side operated. Gentle elevation of the trunk allows more complete drainage. The skull must be trephined in older individuals with closed fontanel. Either Kocher's or Keene's point for trephining may be selected. Kocher's operation is more simple and direct. Like the puncture through the open anterior fontanel, the needle traverses the frontal lobe. The point of selection for trephining is situated 2 1/2 cm. anterior to the central fissure—a point lying somewhat in front of the bregma. The needle should be introduced in a direction slightly downward, backward, and inward to a depth of at least 4 or 5 cm. before the ventricles are reached. At this point the ventricle is broad, extending fully 2 cm. from the middle line; there is practically no risk of hemorrhage during the passage of the needle. After the operation the skin flap is closed over and subsequent punctures are made through the scalp.

Keene's point is preferred by some on account of the better drainage. The site of election is at a point corresponding with the posterior end of the temporal line about 3 cm. behind and an equal distance above the external auditory meatus. At this point the needle enters the posterior part of the first temporal convolution, and should be directed toward the summit of the opposite pinna. At a depth of about 5 cm. the ventricle will be entered at its widest part, that is, where the lateral and posterior cornua are given off from the body of the ventricle at the posterior end of the thalamus.

As a rule, the communication between the two ventricles remains patent, so that tapping one ventricle drains the other also. Drainage, however, of the opposite ventricle is incomplete, so that better results have been obtained by puncture of both ventricles—one at a sitting.

The condition of hydrocephalus is relieved by the simple removal of fluid. If the fluid is clear and sterile no further treatment is necessary. Puncture of either ventricle should be made daily, every other day, or less often as indicated by pressure symptom. A fine catheter or catgut may be left in the ventricle for drainage. If the fluid be infected and contain meningococci, serum treatment should be administered the same as during the lumbar intraspinal operation. The same technique and precautions must be observed during this operation as during the intraspinal operation. The injection of moderate doses of serum is very well borne.

The condition of posterior basic meningitis is usually a chronic one, and lasts very often a few weeks, so that as many as twenty treatments may have to be administered. The condition is almost hopeless, even with treatment. Treatment, however, must not be deferred on that account. Even 1/2 per cent. of recoveries warrants these therapeutic measures. A few cases of recovery following this method of treatment have occurred. Fisher (11) reported one case in 1910. In 1912 two cases recovered, one in Fort Worth and the other in Kansas City.

Some have advised gentle irrigation of the ventricles with saline solution before injecting the antimeningitis serum. The writer has employed
this in some cases, and sees in it little or no advantage. In most instances the fluid is clear and sterile, and in others it is only slightly purulent and flows freely through the needle. Complete drainage, therefore, is easily attained by puncture and little gained by irrigation.

Haynes (16) has conceived the idea of treating certain hydrocephalic conditions by draining the fluid from the hydrocephalic cavity into one of the easily accessible sinuses, attempting to reproduce the course of the fluid into the blood stream. The operation termed by him cisterna, sinus drainage, seems to be based on careful experimental and clinical observation and is worth trying in cases of posterior basic meningitis, where the more simple methods do not give immediate encouragement.

Dangers of the Intraventricular Puncture.—Two dangers must be considered: injury to the vital centers and hemorrhage. As a rule, neither danger need be feared if care be employed to follow the technique described. Danger of hemorrhage lies principally in injury of the pial vessels or the choroid plexus. Puncture at either Kocher's point, Keene's point, or through the lateral border of the open anterior fontanel may cause hemorrhage, rarely severe bleeding. Hemorrhage with subsequent localized palsy, however, sometimes occurs in spite of all precautions. Neither this danger nor injury to vital centers is sufficiently imminent to contraindicate the operation. Direct ventricular puncture is the only hope for these unfortunates, and it should always be done.

Case X. A negro child, aged 12 months, was admitted to the hospital one week after the onset of its illness. The child was unconscious, but her eyes were wide open and staring. Opisthotonos was extreme, the head touching the buttock. The sutures were wide open and the anterior fontanel markedly bulging. There were tonic spasms and contractures of the extremities with occasional severe general convulsions. Lumbar puncture yielded dry tap at three different levels. Diagnosis of posterior basic meningitis was made.

The ventricle was then tapped through the right lateral border of the anterior fontanel. About 30 c. c. of purulent fluid which subsequently demonstrated many extra- and intracellular meningococci were removed. Twenty c. c. of serum were injected into the ventricles with little change in blood pressure or the patient's general condition. On the following day the other ventricle was tapped and 25 c. c. of purulent fluid, in which many meningococci could still be demonstrated, were obtained. Serum here also was injected directly into the ventricles, 20 c. c. being well borne. On the third day the right ventricle was again tapped and 20 c. c. of a less turbid fluid removed. It was thought that possibly the left ventricle was not sufficiently well drained through the right ventricular puncture. Leaving the needle in situ in the right ventricle, another needle was introduced through the left lateral border of the anterior fontanel into the left ventricle. About 10 c. c. of fluid were removed. This demonstrated that drainage was incomplete. Serum was then injected into the right ventricle, the needle being left in situ in the left ventricle, it being thought for the moment that possibly the communication between the ventricles was shut off and that it might be desirable to inject serum into the left ventricle also. Twenty c. c. of serum were injected into the right ventricle. After 10 c. c. were injected, fluid began to appear through
the other needle and as the larger quantities of serum were injected into the right ventricle the fluid began to flow freely from the left needle. This proved that there was free communication between the two ventricles. This patient had in all 16 treatments. After the sixth treatment the fluid had entirely cleared. No bacteria could be demonstrated in the ventricular cerebrospinal fluid. Severe sterile hydrocephalus, however, continued and pressure signs were pronounced. In subsequent ventricular punctures the cerebrospinal fluid was removed, but no serum Injected. After the removal of the fluid there was improvement in the patient’s condition for a period varying between 24 and 72 hours, but after that relapse again occurred. For a period of a few days a drain was left in both ventricles. This, however, did not do any good. One month after admission to the hospital the patient died in a severe general clonic and tonic convolution.

This case illustrates posterior basic meningitis as an early complication of acute meningitis. The cerebrospinal fluid in the ventricles was badly infected. The latter promptly improved under serum treatment, but hydrocephalus was unaffected, ultimately causing death.

Case XI. Child, aged 10 months, was stricken with an attack of acute epidemic cerebrospinal meningitis. Treatment was instituted on the second day of the illness and actively continued. After four doses of serum the child was apparently improving and well on the road to convalescence. Instead of rapidly convalescing, as is usual, the child appeared listless, stuporous, and continued to rapidly waste away. The cerebrospinal fluid was clear and failed to show any bacteria. All active signs of meningitis had also disappeared.

Two weeks after the onset of the disease the child lay in a semi-stuporous state, her eyes wide open and staring. Her head began to be drawn back and after a few days she developed severe opisthotonos, the head almost touching the buttocks. Clonic and tonic convulsions appeared and there were persistent tonic spasms of the hands. The fontanel began to bulge again.

Posterior basic meningitis of the sterile type was diagnosed. Lumbar puncture at three different levels yielded a dry tap. A needle was introduced through the right lateral border of the anterior fontanel into the right ventricle; about 45 c. c. of clear, limpid fluid were removed. Examination failed to show any meningococci either in smear or in culture. The right and left lateral ventricles were alternately regularly tapped at 24-, 48- or 72-hour intervals as necessary for a period of two weeks. Each tap was followed by a prompt improvement of many of the symptoms. Opisthotonos became less marked and the child appeared to be able to see again. Convulsions ceased and tonic spasms relaxed. Progressive, rapid emaciation, however, continued, and after three weeks the child expired.

This case well illustrates the usual form of posterior basic meningitis—the type where the infection has totally been destroyed both in the ventricles and subarachnoid space. Hydrocephalus is extreme and persistent, and in most instances resists all treatment.

Author’s Case of Recovery

Child 11 months old was seen by the writer in consultation with Dr. Saulsberry. There was a history of three weeks’ illness conforming in every respect to epidemic meningitis. First lumbar puncture yielded a very large quantity of turbid fluid showing a few meningococci. The
usual serum treatment was immediately instituted. Puncture and serum injection were repeated twice. There was very marked clinical improvement, but still evidence of a pronounced hydrocephalus and a few meningococci could still be found when the parents decided that the child was very much better and opposed further treatment. The writer did not see the child again until 2 weeks later, when Dr. Saulsberry reported that the child was having severe convulsions and he thought was about to die. The child at this time presented a typical picture: a peculiar facies, the eyes open, staring with lids retracted; the face blanc and expressionless, disturbed at times by a grimace accompanied by a shrill hydrocephalic cry. The head was markedly extended and the body showed extreme form of opisthotonos, the head almost touching the buttock. The child did not seem to see and could not swallow. The head was markedly enlarged, the sutures widely separated, anterior fontanelle markedly bulging; the head felt like a bag full of water. There was marked rigidity of the whole body and persistent convulsive spasm of the upper extremities, which were extended with hands clenched and lower extremities with the feet extended and toes flexed. There was a marked tâche cérébrale. Heart action was rapid and intermittent, but at times during the day it was slow and intermittent. Respirations were slow, irregular, with long periods of apnea, breathing corresponding best to the Biot type. Reflexes were markedly exaggerated. The child had been lying in this "hypnotic state" for several days. Occasionally there was explosive projectile vomiting.

The child presented evidence of terrific hydrocephalus; the peculiar facies, the staring eyes, the retracted lids, the extreme opisthotonos suggested that we were dealing with posterior basic meningitis.

Lumbar puncture made at two levels yielded a few drops of cerebrospinal fluid. A ventricular puncture was then done.

The right ventricle was first tapped, 50 c. c. of slightly turbid cerebrospinal fluid was removed under very high pressure and 20 c. c. of serum containing 2.10 per cent. of tricesol preservative was injected. There was a fall of 10 mm. of mercury in blood pressure on the removal of the fluid, but no change on injecting the serum. The next day the condition was somewhat improved, convulsions were controlled, but other pressure signs were again present. The left ventricle was tapped and 80 c. c. of slightly turbid cerebrospinal fluid was again removed and 20 c. c. of serum injected with blood pressure change as on the previous day. The cerebrospinal fluid obtained the first day showed a moderate number of pus cells and a few meningococci, extra- and intracellular. The second fluid showed only intracellular meningococci.

Two days later the right ventricle was again tapped; 60 c. c. of clear fluid was removed. No meningococci were found. The child showed considerable improvement though he still did not seem to see, opisthotonos
less marked, pulse and respiration less irregular. The child nursed, had no temperature, cried more normally and in general was much improved.

The parents again decided against further treatment. The child continued to improve, and two weeks after the last puncture all the pressure signs and opisthotonos had disappeared, the child could see, and nutrition improved.

I did not see the child until two months later when Dr. Saulsberry called me on account of attacks of dyspnea and cyanosis with loss of consciousness, which first appeared one month previously, coming on at intervals of a few days but in the past week several times daily.

The child presented a most astonishing picture. He was bright, stout, and had grown tremendously. In two months he had put on the growth that usually requires a year and a half. He was active, playful, could see well, and had developed mentally almost in proportion to his skeletal growth. The head was large and showed signs of hydrocephalus. There was also evidence of a large thymus. I interpreted the condition as due to a persistent hydrocephalus and attributed the excessive skeletal development to a possible pituitary involvement. Lumbar puncture was performed and 60 c. c. of clear fluid was removed. The puncture proved that the communication between the ventricles and subarachnoid space had been reestablished. The child did not have convulsions until three days later. For the next two weeks convulsions occurred once every few days and seemed to be generally improved after the puncture.

The last the writer heard of the child, six weeks later, the convulsions were less frequent and there was continued good development.

This case is of importance as demonstrating the value of therapy. The recovery was complete with the exception of subsequent convulsive seizures. The complicating hyperpituitarism and thymic growth were of unusual interest. It also shows that communication between ventricles and subarachnoid space can be reestablished. This may be explained when the closure is due to an inflammatory exudate.

**GENERAL TREATMENT OF MENINGITIS**

The fact that one is dealing with delirious and unconscious patients renders the general treatment important.

The general nutrition should have careful attention. Fluids and nourishment should be given abundantly. A liberal soft diet is well borne.

The bowels, which tend to be constipated, should have careful attention. Laxatives and enemas should be used as necessary.

The bladder needs special attention. Paresis, with loss of control of the vesical sphincter, is common. In most instances the apparent incontinence which is recorded is really an overflow of a little urine from an
overdistended bladder. Restless and delirious patients will often become quiet and sleep after catheterization. It is a safe rule during the period that the patient is irresponsible to order regular catheterization of the bladder.

Restlessness and delirium are very much benefited by the ice helmet and warm sponging. It is questionable whether or not the ice helmet has any virtues other than its sedative action.

The usual sedatives of bromid, chloral, combinations of phenacetin, aspirin and codein, and codein and veronal are usually necessary during the acute period of the disease. Morphin is often imperative. Sometimes hyoscin must be used.

The pain and general restless symptoms after puncture with injection of serum are benefited by the local application of ice bags or hot-water bags at the site of the puncture. Raising the head of the patient will often relieve the headache and vertigo which quite often follow. Morphin is often necessary during or immediately after the operation.

Some workers have recommended that the foot of the bed be raised about twelve inches, the purpose being to aid the better circulation of the injected serum. This procedure is often followed by complaint of headache and vertigo. Furthermore, the writer has found nothing gained by this expedient, judging by the comparative studies of cases in which it has been employed as against those in which the patient has been kept level.

**Internal Medication.**—Some observers have demonstrated that urotrpin, taken by mouth, is secreted into the cerebrospinal fluid, where it has some disinfecting properties. Urotrpin alone, without other treatment in epidemic meningitis, is not curative, but as an aid has some value. Large doses should be prescribed; not less than 40 to 60 gr. daily administered in large quantities of water are advised.

[The irritant action of urotrpin on the kidneys should be borne in mind. If hematuria develops the drug may be temporarily withdrawn.—Editors.]

**General Treatment of Convalescents.**—Patients must not be allowed out of bed too soon on account of the persistent hydrocephalus. Iodid internally, iron and other tonics, together with liberal diet, help.

**Treatment of Complications**

The complications of meningitis are many and dangerous. They may be grouped into two large divisions. In the one are included all those complications resulting directly from the local cerebrospinal inflammation with destruction of tissue, including changes in mentality, various paralyses, eye complications, and deafness. The second division consists of those
complications due to the complicating general meningococcus sepsis present before and during the course of meningitis. This includes the common joint complications, septic pneumonia, pyelitis, general meningococcus sepsis, meningococcus endocarditis, middle ear infection, some of the eye infections, phlebitis, and neuritis.

Treatment of Changes in Mentality

The commonest change in mentality following meningitis consists in a change from an amiable, pleasant personality to one that is irritable, vicious, and unreliable. These changes most often clear up spontaneously without special treatment. Sometimes mild, persistent, simple hydrocephalus is the cause. In these cases puncture with relief of pressure helps considerably.

Temporary or persistent imbecility is fortunately a less common complication. These cases offer much less hope of improvement. Persistent hydrocephalus, however, in these cases, as in the former, is very often an important influence. Careful examination, therefore, should always be made for evidence of hydrocephalus and, if present, simple lumbar puncture should be done and fluid removed.

The most severe and dreaded complications under this heading are the cases of severe meningomyelo-encephalitis. The clinical picture is one of lingering, absolute imbecility, with stupor, paralyses, occasional convulsions, incontinence, gradual wasting, with ultimate dreadful emaciation, bed sores, and finally death after a period of weeks, months, even a year. The pathological condition is one usually of meningomyelo-encephalitis, with moderate hydrocephalus. Treatment should be along the following lines: (1) occasional tap, with relief of hydrocephalus; (2) occasional injection of serum if a mild, persistent infection be present; (3) the use of meningococcic vaccine. These cases, however, offer but little hope; almost all die.

Paralyses Complicating Meningitis

This is one of the more frequent complications. Most often the palsies are cerebral in origin, and consist of mono- or hemiplegia. Less often palsies are peripheral in origin. The latter sometimes are the direct result of lumbar puncture—high lumbar puncture with injury of some of the centers of the cord. In either instance the prognosis is fair, especially in young individuals. There is no specific treatment. The same general measures should be employed as in paralysis from other causes.

Eye Complications

The great frequency of eye complications in meningitis may possibly, to a very great extent, be explained by the structure of the optic nerve and its intimate association with the brain. It is frequently described by
anatomists as a prolongation of the brain substance rather than as an ordinary cerebrospinal nerve. As it passes from the brain it receives sheaths from the cerebral membrane, a perineural sheath from the pia mater, an intermediate sheath from the arachnoid, and an outer sheath from the dura mater, which is also connected with the periosteum as it passes through the optic foramen. These sheaths are separated from each other by spaces that communicate with the subdural and subarachnoid spaces respectively. The innermost or perineural sheath sends a process around the arteria centralis retinae into the interior of the nerve, and enters immediately into its structure. Thus inflammatory affections of the meninges or of the brain may readily extend along these spaces or along the interstitial connective tissue in the nerve.

The intimate association between the infected meninges and the ocular nerve may thus readily explain the frequent eye suppurations in epidemic meningitis.

Another possible mode of infection in meningitis may be the severe general bacteriemia (sepsis) which is frequently present in the acute stages of the disease.

The most common eye complications in the order of their frequency are conjunctivitis, palsies, suppurative choroiditis, and infection of any of the other structures of the eye or panophthalmitis.

Conjunctivitis is a very early complication, sometimes even occurring in the premeningitic stage. The condition is benign and usually heals spontaneously. Little treatment is necessary.

Crops of herpes on the eyelids and cornea are very occasionally seen.

Eye palsies, most often of the sixth nerve, causing strabismus, are temporary and spasmodic in character. Permanent paralyses of this nerve or of the third nerve are very rare.

Suppurative choroiditis, or infection of any of the other structures, or panophthalmitis, should be diagnosed early and the regular treatment promptly instituted. These complications, unfortunately, are quite common in meningitis, and are the most frequent causes of blindness.

The local application of antimeningitis serum here again suggests itself. The action of the antimeningitis serum following its local subdural injection has already been explained. The serum benefits principally through its local opsonic action while bathing the parts and stimulating the leukocytes to digest the bacteria. The same reaction occurs in test tubes or in injections into the peritoneal cavity of the guinea-pig of live culture of the meningococcus and the specific serum. The local application of the antimeningitis serum in the eye will, therefore, suggest itself as a rational measure. In cases of conjunctivitis it certainly ought to be very beneficial. Fortunately, however, these cases clear up spontaneously and do not require any special treatment. Observations on the effect of serum locally applied should nevertheless be made. Serum used
early in cases of conjunctivitis may prevent the severe complications of conjunctivitis occasionally encountered, and may possibly avert or benefit the cases with deeper infection.

The other forms of blindness are due either to pressure or nuclear lesions. Pressure is one of the rarer causes, and is seen in the forms of extreme hydrocephalus, as well illustrated in cases of posterior basic meningitis where the ventricles are markedly distended with the encapsulated exudate. A study of the fundus shows a decided blanching of the vessels, immediately followed by their filling up, with temporary improvement in vision after ventricular drainage. Other cases show a varying degree of optic neuritis.

Prognosis in cases of blindness following nuclear lesions is bad, and little or nothing can be done.

**Ear Complications**

Middle ear suppuration and deafness are the principal ear complications. Middle ear suppuration usually remains localized, rarely extending deeper. Quite often the meningococcus can be demonstrated in the purulent discharge early in the infection. The usual treatment of paracentesis and drainage suffices.

Deafness, unfortunately, is not only one of the most dangerous, but one of the most common complications of meningitis. A small percentage of cases recover. These are probably principally caused by hydrocephalus, and with the subsidence and relief of this condition deafness clears up.

This temporary deafness is not infrequently seen during the course of chronic meningitis. The condition is relieved after each puncture and recurs as the cerebrospinal fluid reaccumulates.

*Case XIII.* A man, aged 47, had suffered from epidemic meningitis for two months. After the acute stage of the disease he had lapsed into the chronic form, the disease conforming to the milder type of chronic epidemic meningitis, hydrocephalus being the principal feature, and mild, persistent infection apparently being of less consequence. With the periodic occurrence of hydrocephalus, the patient began to complain of buzzing, roaring noises in the head and of severe deafness. With the relief of hydrocephalus by lumbar puncture, deafness promptly cleared up. After an illness of two months the patient had sufficiently recovered so that he could get about. He, however, complained of fairly persistent headache and considerable impairment of hearing. The pupils remained dilated and he suffered from occasional moderate diplopia. The veins of the scalp were moderately dilated; Mac ewen's sign was quite pronounced. The symptoms were considered to be due to hydrocephalus, and lumbar puncture was advised but declined by the patient. These symptoms persisted for a period of about six weeks, gradually improved, and ultimately disappeared. The patient's hearing was finally as good as ever.

*Case XIV.* A boy, aged 8, was admitted to the hospital suffering from a very severe acute attack of epidemic meningitis. Twenty-four hours later it was noted that he was completely deaf. Under the course of the usual active serum
treatment he promptly improved and was convalescing on the tenth day after admission to the hospital. On the fourteenth day he was discharged. He was able to get about and felt well in every way; no headache; no evidence of hydrocephalus. He was absolutely deaf in both ears, however. When seen six months later there was no improvement. Deafness in this case was evidently due to earlier nuclear lesions.

In all cases of deafness, therefore, it is most important to determine whether or not hydrocephalus is present, since this offers practically the only hope of relief.

The other more common permanent form of deafness occurs often soon after the onset of meningitis, and is due to the destruction of the auditory apparatus. The auditory nerve, like the optic nerve, is very closely associated with the meninges. It is generally believed that the infection in the meninges extends along the arachnoid sheath of the auditory nerve into the auditory canal, spreading along the vestibular nerve and infecting the structures of the inner ear. With recovery from the primary disease the auditory nerve degenerates, and a cicatrix fills up the internal auditory canal and the structures of the internal ear. This form of deafness is independent of hydrocephalus, and is not amenable to treatment.

Joint Complications

Under the division of complications due to general meningococcus sepsis the joint complications will first be considered. Joint involvement occurs in 15 per cent. of all cases, appearing during all stages of the disease. In most instances it is a polyarthritis affecting the smaller joints of the hand and the larger joints of the upper and lower extremities. The lesion is usually benign and clears up spontaneously.

The usual acute form of arthritis occurs very early in the disease and consists of a mild, only moderately painful synovitis, which subsides without any special treatment in a very few days. Sometimes, however, the condition tends to become a chronic one, lasting weeks or months. The joints are painful and swollen, the tissues thickened, and there is moderate disability. The condition is benefited by local measures of heat, massage, and counter-irritants. Meningococcus vaccine offers most hope of permanent and rapid cure. Small doses of 50- to 100,000,000 meningococci should be used at first, later followed by larger doses, until response is observed or the reaction is too severe. The doses should ordinarily be administered at intervals of 3 to 5 days, but it is best to be guided by the response and the reaction.

A less common form of this complication met during the course of meningitis is a very severe acute arthritis. The joints are severely swollen and painful. The condition is accompanied by high septic temperature. Instead of clearing up quickly, as does the usual form, this condition tends to become more aggravated. Active radical measures are indicated. It
has been found that tapping the joints and removing the fluid in them by aspiration, followed by the injection of a small dose (10 to 15 c. c.) of antimeningitis serum directly into the joint cavity, give prompt relief and sometimes brilliant recovery. Response is immediate and even more gratifying than in the subdural treatment with the antimeningitis serum. Swelling and all evidence of local inflammation usually promptly subside. This is another instance of the rational and beneficial effects under the direct, local application of specific immune sera to the infected site.

**Septic Pneumonia**

Septic pneumonia is one of the more frequent terminal complications. The principal treatment is prophylactic. Delirious, stuporous patients should be turned from side to side to prevent hypostasis. The throat and mouth should be kept clean, and care should be used while feeding a patient to prevent inhalation of food.

**Pyelitis**

In epidemic meningitis, as in other forms of general sepsis, pyelitis is quite common, and is evidenced not only by a bacteriuria, but also by the appearance of pus and casts in the urine. During the bacteriemic, premeningitic stage one can frequently find meningococci and pus cells in the urine in considerable numbers even before meningococci can be found in the cerebrospinal fluid. With the localization of the infection in the meninges and the appearance of meningococci in the cerebrospinal fluid, the organisms and pus cells either diminish very considerably or totally disappear from the urine. This, of course, indicates that the general infection has subsided to a marked degree. Occasionally, however, pus cells and meningococci persist in the urine, and may be accompanied by tenderness and enlargement of the kidney. This condition of pyelitis rarely, if ever, goes on to surgical kidney. It is important to recognize the condition, since sometimes one can explain persistent fever which otherwise might be attributed to the meningitis proper. No special treatment is necessary. The general measures of urotropin and active elimination suffice.

**Heart Complications**

Chronic meningococcic sepsis due to localization of the meningococcus during the period of general sepsis in any of the valves of the heart with the production of a chronic, ulcerative, or malignant endocarditis is a very rare complication. The picture is the usual one of malignant endocarditis. The cases linger from a few weeks to several months. Anemia and emaciation are progressive and infarctions more and more frequent. There is very little hope in treatment. The antimeningitis serum injected subcutaneously and active vaccination, preferably with an autogenous menin-
gococcus vaccine, offer most hope. The antimenigitis serum should be
injected at first in moderate doses of 20 to 40 c. c., repeated at intervals
of a few days. If there be no response after a few doses active vaccination
should be instituted, beginning at first as in other cases with small doses,
repeating the doses at frequent intervals and increasing the dose until
response is apparent. These cases are so rare that early diagnosis is usually
missed. Active specific treatment with serum and vaccine should offer a
fair percentage of recoveries if treatment be instituted early.

Case XV. Boy, aged 13, after six days’ serum treatment of epidemic menin-
gitis was apparently making a brilliant recovery. His cerebrospinal fluid had quite
cleared up; temperature had come down to normal and all clinical signs of mening-
gitis had abated. After 24 hours of normal temperature fever again rose to 104
degrees. The first suspicion, of course, was that the patient was suffering a re-
lapse. He, however, presented no clinical symptoms of a relapse. A careful ex-
amination led to the discovery that the patient had a tender, slightly enlarged
kidney on the right side. The urine had a moderate number of pus cells and
Gram-negative diplococci which subsequently culture proved to be meningococci.
Urotropin was administered in large doses. He continued to run a septic tempera-
ture, fluctuating between normal and 104 to 105 degrees daily. In order to elimi-
nate absolutely the possibility of a slight, persistent infection in the meninges,
another lumbar puncture was done on the second day after the reappearance of
these symptoms. The cerebrospinal fluid, however, was normal in every way.
After a period of 10 days temperature came down to normal and with it there
was a coincident clearing up of the tenderness and enlargement of the right kidney.
The pus cells and bacteria disappeared from the urine.

Serum Sickness

While serum sickness proper is not a complication of meningitis, it is
so commonly seen during the course of meningitis that it may be properly
classified as one of the common complications of the disease.
The writer has noted the complication in about 60 per cent. of 300
cases which he has personally observed. The antimenigitis serum is not
refined or concentrated like diphtheria and tetanus antitoxin; and very
large doses must be used. An average case is injected with 100 to 200 c. c.
of serum during the course of the illness. Absorption of the serum into
the general circulation is very rapid after its injection into the subarach-
noid space; in meningitis absorption is even more rapid on account of the
large area of inflammatory tissue with which the serum comes into direct
contact.

Symptoms usually appear on the eighth to tenth day after the first dose
of serum. Not infrequently the accelerated reaction occurs on the fourth
to sixth day after the first injection in cases where the dose of serum has
been repeated. The writer has also seen a number of cases where the im-
mmediate reaction occurred within a few minutes after the first dose of
serum. In a few cases the history of sensitization to horse serum through
previous injection with diphtheria antitoxin was obtained. In 6 cases,
however, there was apparently constitutional sensitization to horse serum; no previous sensitization to horse serum had been produced. These cases conform to those which have been discussed following the first dose of diphtheria antitoxin.

The symptoms are, in the majority of cases, annoying, but not alarming, conforming in every way to the well-known picture of serum sickness and consisting of marked general giant urticaria, or a general erythema, erythema multiforme, or occasionally angioneurotic edema. There are some nausea and vomiting and moderate fever. Pain in the joints, sometimes accompanied by slight swelling, albuminuria, and general adenitis of moderate severity occasionally occurs. In the average case the patient complains of severe itching, which is very annoying and resists almost every means of treatment. It rarely lasts, however, more than 12 to 24 hours.

Occasionally the symptoms above described may be much more severe and, for a time, may be very alarming, especially if the appearance of urticaria be delayed, and if there be doubt as to the causation of the symptoms. The patient may have a severe chill and develop a very high temperature; may become prostrated and sometimes may suffer severely from shock. These cases may be alarming and may even terminate in death. The following case (quoted from Sophian, "Epidemic Cerebrospinal Meningitis," St. Louis) illustrates:

Case XVI. Moderately severe cases of epidemic meningitis. Injected with serum on three consecutive days following patient's admission to the hospital, then on the fifth day, and tapped for simple removal of fluid on the seventh day. Symptoms were very much improved, child was brighter, stiffness of the neck was only slight, the Macewen was slight, temperature was 100° F., and cerebrospinal fluid had cleared up, yielding only a few intracellular organisms. On the eighth day temperature suddenly rose to 104° F. General condition was not so good. The patient vomited, appeared stupid, pulse was weak, but there were no other pressure signs. The onset of such violent symptoms in the face of previous steady improvement caused the author to suspect that possibly the meningitis was not accounting for the symptoms. General treatment was given. About two hours later a marked urticaria appeared all over the body. General condition became worse and pulmonary edema quickly developed. It was noticeable, however, that, while the general condition was not good, it was not as bad as it would be usually with terminal pulmonary edema. Active general treatment with cupping of the chest gave immediate response in a few hours. The following day urticaria was still present, but general condition was good and edema entirely gone.

During the course of treatment of an acute case of meningitis the development of these symptoms may be very confusing in that there may be doubt as to whether the severe general symptoms and high fever are due to a relapse of meningitis or to the serum sickness or other complications. If the patient be still suffering from meningitis, there may appear an aggravation of some of the meningeal symptoms, especially the stupor, headache and rigidity of the neck. If the patient be convalescing from meningitis, there likewise may appear a group of meningeal symptoms which may lead to the suspicion that the patient has suffered a severe re-
lapse. Netter and Debré have described a group of cases in which severe meningeal edema was the predominating feature of the attack of serum sickness. Clinically the symptoms were very suggestive of a relapse. Lumbar puncture yielded a clear fluid with no organisms.

The appearance of the above-described untoward group of symptoms occurring on the eighth to tenth day of the disease in a patient who apparently has been doing well, and who is convalescent from meningitis, should always lead to the suspicion of serum sickness, even though the urticaria proper has not yet appeared. If the meningeal condition has been responding as well as can be expected it is well to leave the patient alone, rather than to puncture unnecessarily and further depress him.

In the writer's experience almost any secondary complication during the course of meningitis which is accompanied by fever is usually promptly attended by an aggravation of the meningeal symptoms, especially in the rigidity of the neck and Kernig's sign, even though lumbar puncture does not reveal an actual relapse or aggravation of the meningitis proper. For example, one of the writer's cases, a girl of fourteen, developed repeated crops of herpes, the last crop occurring on the eighth day of the disease when the patient was convalescing from meningitis. Each crop of herpes was attended by a rise in temperature to 104°, and each crop, furthermore, even the last, was attended by increased rigidity of the neck, stupor, and marked Kernig. It is possible that the cases described as meningeal edema complicating serum sickness, might be explained in this way.

To recapitulate: On the suspicion of serum sickness it is well to leave the patient alone and treat him generally. Under no circumstances, however, should serum be administered under the impression that the patient is having a relapse if a strong suspicion points to the symptoms being due to serum sickness proper.

The great frequency of serum sickness following the injection of the antimenningitis serum should render one cautious in administering the serum by intraspinal injection if there be an interval of several days since the last dose of serum. The following instance of anaphylaxis (quoted from Sophian's "Epidemic Cerebrospinal Meningitis," St. Louis) illustrates this danger:

Case XVII. Girl, aged 10. Moderately severe case of epidemic meningitis. Had been injected with the antimenningitis serum subdurally on two successive days with considerable improvement so that the attending physician thought that further serum treatment might be unnecessary. Four days later (6 days after the first dose of serum) a moderate relapse was observed and the patient was sent to the hospital. Her general condition was very good. Lumbar puncture was performed and 15 c. c. of antimenningitis serum were administered. Her condition at the end of the operation was good. Four hours later a severe, giant urticaria suddenly broke out, accompanied by delirium and symptoms of intense shock. Pulse was rapid and weak; color was cyanotic, and within an hour a severe general pul-
monary edema developed. The immediate condition of meningitis was not aggra-
vated. Active general treatment fortunately brought notable response in a few
hours. The patient made an uneventful recovery from her meningitis.

The writer, in a very large experience, has never had a fatality as a
result of anaphylaxis. Such fatalities, however, have been reported by
other observers. Besredka (3), in calling attention to the great frequency
of serum sickness following the use of the antimeningitis serum by intra-
spinous injection, mentions 10 fatal cases.

Rosanow (13) has advised the preliminary injection of the serum
subcutaneously, intramuscularly, or intravenously in doses of .4 to 2 c. c.
as a protection against the anaphylaxis following the intravenous injection
of serum. (The same would hold true for the intraspinal injection.)

The case quoted by Grysez and Dupaich (9), in which a preliminary
intraspinal injection of 2 c. c. of serum given a chronic case of meningitis
(the last dose of serum had been three weeks previous), did not prevent
the occurrence of severe symptoms of anaphylactic shock following the
injection of the therapeutic dose of serum, shows the unreliability of this
method also.

Besredka (2) goes a step farther by suggesting that a patient may be
protected by the preliminary injection of repeated, instead of single, doses
of serum. These should be applied at short intervals, the dose being
gradually increased. This method of desensitization appears to be more
effective.

Weil in a recent publication, shows by observation on guinea-pigs
that the desensitizing dose varies in proportion to the initial sensitizing
dose; where the sensitizing dose was small the desensitizing dose should
be small and vice versa. He calls attention to the obvious difficulty of
determining the necessary desensitizing dose for the human being and,
therefore, the impossibility of absolutely safeguarding a patient by either
the injection of a single large dose of serum or by the repeated doses. The
use of the very large therapeutic doses of serum in meningitis would re-
quire very large desensitizing doses of serum injected subcutaneously.

An analysis of this subject warrants the following deductions:

Serum sickness, though of frequent occurrence following the injection
of antimeningitis serum, is rarely fatal.

It is desirable to inject a protective desensitizing dose of serum if there
be an interval of several days between the doses of serum.

The most practical desensitizing protective method at the present time
is the subcutaneous injection of a few cubic centimeters of serum a few
hours before the intraspinal dose. The complication of true anaphylaxis
terminating fatally is so rare that one is not justified in withholding the
therapeutic dose of serum on that account.

Treatment of Serum Sickness.—All treatment is concerned with the
relief of the extreme itching and in the case of severe symptoms with general treatment for shock. The local sedatives of value are alcohol, warm sponges, sometimes ice-cold sponges, the use of bicarbonate of soda, menthol, anesthesin ointment, and other well-known local sedatives. Internally general laxatives should be taken; diet should be light. Salol and menthol appear to help, and sedatives, such as codein or, if necessary, morphin, or atropin in 1/100 gr. doses, sometimes seem to shorten the duration of the attack.

For anaphylaxis general measures of active stimulation, artificial respiration, if necessary, or chloroform for convulsions should be used.

In case of relapse serum treatment would be indicated; in case of serum sickness general treatment. If serum be injected by mistake in the latter condition the danger of "immediate" serum reaction or true anaphylaxis would complicate the existing condition. The general experience with serum treatment, however, is that neither reaction at such a time would be apt to ensue.

**Relapse**

Relapse is more common than it should be with proper treatment. The principal cause is a discontinuation of serum treatment before the infection has been sufficiently controlled. One or more doses of serum injected subdurally may give such marked and prompt response that the physician is often tempted to leave well enough alone, even though the patient shows some sign of persistent infection and hydrocephalus. This combination of persistent hydrocephalus with mild infection is most dangerous, since it not only has a tendency to cause chronic meningitis, but also to invite the serious complications previously described. Thus, in many instances, cases of relapse are not relapse at all, but rather an aggravation of cases of chronic meningitis, an aggravation of the hydrocephalus and a lighting up of the infection in the meninges, which infection had only partially been destroyed. Cases such as these should properly not be classified as relapse. The patients had never really recovered.

**Treatment.**—The first essential in treatment is prevention. Serum treatment in cases of epidemic meningitis should be continued as long as is necessary; that is, until all trace of active infection has disappeared and all evidence of severe persistent hydrocephalus has been eliminated. If bacteria be present in the cerebrospinal fluid, and especially if they be extracellular, serum treatment should be continued.

Treatment of relapse proper should be carried out along the same lines as described for the acute condition. Indications for the doses of serum and for relief of hydrocephalus are the same as for acute meningitis. Vaccine, especially autogenous, in this condition helps more promptly to clear up the infection.
ANALYSIS OF INFLUENCES AFFECTING PROGNOSIS

Prognosis of all infections depends upon the same factors: (1) the severity of the infection; (2) the resistance of the patient; and (3) the character of the treatment employed.

The mortality rate (70 to 90 per cent.) of cases not treated with serum speaks for the severity of the infection in meningitis. The fulminating, severe bacteriemic cases offer the worst prognosis. These often die before any treatment can be instituted. The average acute case offers best hope of response to serum therapy if treatment be instituted within 2 to 3 days after the onset of the disease. The prognosis of the chronic meningitis cases is much worse; many more die, and those recovering often have serious sequelæ.

The prognosis of the posterior basic meningitis cases is uniformly bad.

The most important factors in the resistance of the patient are the age and general condition of health. Age incidence is an important influence, most probably on account of the ability to resist infection. Prognosis in children under one year of age is very bad. Fully 50 per cent. die, even with early instituted specific serum treatment. Likewise the prognosis in old people is not so good. Robust individuals in good health have, of course, a better prognosis than weak individuals. The prognosis is especially poor among alcoholics, who have a tendency to develop violent, exhausting delirium and early hypostatic pneumonia.

<table>
<thead>
<tr>
<th>Table of Age Mortality ¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Under 1 year ............</td>
</tr>
<tr>
<td>1 to 2 years .............</td>
</tr>
<tr>
<td>2 to 5 years .............</td>
</tr>
<tr>
<td>5 to 10 years ............</td>
</tr>
<tr>
<td>10 to 20 years ...........</td>
</tr>
<tr>
<td>Above 20 years ..........</td>
</tr>
</tbody>
</table>

¹ From Sophian's "Epidemic Cerebrospinal Meningitis," St. Louis.

The most important influence affecting prognosis in meningitis is treatment. Treatment in turn is most influenced by the early diagnosis, the use of a potent, highly immune serum, the proper administration of serum, and the active administration of treatment until the infection is thoroughly under control. Early diagnosis is most important. Statistics recorded by all authors under the best form of serum treatment confirm
that the most successful results are obtained when treatment is begun on the first to third day of the disease; next best, when treatment is begun on the fourth to the seventh day of the disease, and worst results when treatment is instituted later in the disease. The following table graphically bears this out:

**Mortality per Cent.**

<table>
<thead>
<tr>
<th>Day of the Disease When Treatment Was Begun</th>
<th>Flexner's Cases (712)</th>
<th>Dopter's Cases (402)</th>
<th>Netter and Debre's Cases (99)</th>
<th>Author's Cases (180)</th>
<th>Author's Corrected Statistic Cases (161)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First to third day</td>
<td>25.3</td>
<td>8.20</td>
<td>20.9</td>
<td>13.0</td>
<td>9.0</td>
</tr>
<tr>
<td>Fourth to seventh day</td>
<td>27.8</td>
<td>14.40</td>
<td>33.3</td>
<td>25.6</td>
<td>14.9</td>
</tr>
<tr>
<td>Later than seventh day</td>
<td>42.1</td>
<td>24.10</td>
<td>28.0</td>
<td>37.1</td>
<td>22.6</td>
</tr>
<tr>
<td>Average mortality</td>
<td>34.1</td>
<td>16.44</td>
<td>28.0</td>
<td>25.0</td>
<td>15.5</td>
</tr>
</tbody>
</table>

1 From Sophian's "Epidemic Cerebrospinal Meningitis," St. Louis.

The importance of a highly potent antimeningitis serum is apparent. Unfortunately there is no accurate method of standardizing the antimeningitis serum. The standard for diphtheritic and tetanus antitoxins is uniform so that the Federal Government can check up the products offered on the market and prove whether or not a product contains the required number of immune units. The methods used in determining the potency of a serum, consisting of the opsonic test, bactericidal test, complement-fixation test, all depend, to a very great extent, upon the personal equation and the reagents used in the test so that a uniform standard in terms of opsonic units or complement-fixation cannot be established. Variations and fluctuations in potency of different preparations of the antimeningitis serum can, therefore, be readily understood. This, without a doubt, explains some of the poor results recorded at different times in treatment. All manufacturers of antimeningitis serum should carefully check up the potency of their product, not only by laboratory biological tests, but by carefully watching the results of clinical tests as well, since by the latter observations alone can one be absolutely certain whether or not a product is up to the desired potency.

The importance of properly administering the antimeningitis serum has been fully explained in the preceding pages. It must always be borne in mind that incorrect technique may be both harmful and very dangerous—harmful in that the patient is temporarily depressed after the injection of the serum, allowing the infection to temporarily gain headway, and dangerous in that the patient may be killed as a direct result of improper injection. The patient should always be carefully watched during the operation, and blood-pressure observations should always be made.
INFLUENCES AFFECTING PROGNOSIS

The importance of properly following up the serum treatment is now also apparent. Dangerous chronic forms of meningitis and posterior basic meningitis will, to a very marked degree, be prevented, and many of the dangerous complications and sequelae will be avoided. Treatment must always be actively kept up as long as bacteria persist in the cerebrospinal fluid in any numbers, the exception to this being where there is a prompt response under serum treatment and a few intracellular bacteria persist in the cerebrospinal fluid. With accompanying good clinical condition one is in these cases warranted in waiting 24 hours, or possibly a little longer, before treatment is again administered.

Subjective and objective symptoms of hydrocephalus must also be carefully watched, and, if persistent, hydrocephalus must be treated by simple puncture with removal of fluid. Cases of posterior basic meningitis should be recognized early and direct intraventricular puncture be performed at the earliest moment. This is the only possible hope for these cases, and active intraventricular treatment should in all cases be instituted and kept up as long as there is any hope.

It has been demonstrated by the writer and others that the meningococcus is made up of a number of strains, as differentiated by immune serum tests. This difference in strains explains why some writers believed that posterior basic meningitis was produced by an organism differing from the meningococcus, and also explains such classification as the parameningococcus.

It is very probable that an epidemic in a community is produced by the same strain of meningococcus. Occasionally some cases of only moderate severity resist the serum treatment, even though it be instituted early and under favorable conditions. Such failure can be explained by the causation of the disease by a strain not included in the immune antimeningitis serum employed.

The most valuable signs of response to serum treatment are the effect upon the sepsis and clearing up of the hydrocephalus. The effect upon the fever is especially striking. About one-third of the cases show favorable response by critical fall in temperature, and many others by gradual fall to normal by lysis a few days after serum treatment is begun.

Prompt improvement and rapid clearing up of violent delirium, stupor, and convulsions are likewise very remarkable. A not uncommon picture is a violently delirious, restless, noisy patient one day and 24 hours later, after serum treatment, a rational, quiet, sleeping patient.

General improvement and clearing up of active signs of meningeal inflammation, as evidenced by improvement in the Kernig sign and neck rigidity, often go hand in hand with clearing up of the active mental symptoms.

The most convincing sign of improvement, however, is demonstrated by microscopic examination of the cerebrospinal fluid. The change in the
sediment after one dose of serum from the picture of many bacteria, mostly extracellular, to a microscopic picture 24 hours later of few bacteria mostly intracellular, is absolutely convincing. As a rule there is coincident macroscopic evidence of improvement in the clearing up of the turbid cerebrospinal fluid. This alone, however, is often misleading. At times the cerebrospinal fluid becomes much more turbid after a dose of serum, even with marked improvement. This may be explained by referring to the action of the antimeningitis serum. The serum acts principally through its local stimulation of leukocytosis and phagocytosis. Thus sometimes the fluid becomes more turbid on account of increase in polymorphonuclear leukocytes, but microscopic examination shows few bacteria, and those intracellular.

Improvement in hydrocephalus, as has been explained, is not ordinarily as prompt as the subsidence of the infection. A rapid clearing up of hydrocephalus, as shown by the diminution in the quantity of the cerebrospinal fluid obtained by lumbar puncture, is especially gratifying. We must reiterate, however, that total diminution in quantity of fluid alone does not mean improvement, since sometimes symptoms of sepsis are much aggravated, even though the quantity of exudate be less. The possible onset of posterior basic meningitis indicated by the small quantity of fluid obtained by lumbar puncture must always be borne in mind.

The most important immediate effect of proper serum treatment is in shortening the period of the illness and in the effect on prognosis. Before the days of serum treatment the disease was either rapidly fatal or long drawn out, and finally fatal in the majority of cases. The few cases of recovery rarely lasted less than one week. Fully 50 per cent. were drawn out over 5 weeks or longer. As a significant contrast is the recovery of most cases in the short period of 1 to 2 weeks, many cases clearing up absolutely in 5 to 6 days after the beginning of treatment.

The comparison of mortality statistics in cases treated without serum and those treated with serum is very interesting. The writer's personal experience in the Texas epidemic of 1912 is very significant. During the months preceding his arrival in Texas there were about 105 cases in Dallas and the immediately surrounding country. The mortality among these cases was fully 90 per cent. Some of the few reported cases of recovery were later treated by the writer for relapse, chronic hydrocephalus, posterior basic meningitis, and other complications. On account of the previous scarcity of serum most of the 105 cases had not had the benefit of full serum treatment. A large proportion of those who had been treated with serum had not had the advantage of repeated injections. During the months following his call to Dallas the writer treated personally 180 cases with a gross mortality rate of about 16 per cent. Dr. Steiner, president of the State Board of Health of Texas, collected during this epidemic a total of 2,280 cases in the State. The mortality among the serum-treated
cases was 37 per cent. as against a mortality of 77 per cent. among those not treated with serum. Complications among the recovered serum-treated cases were relatively few as against the complications among the recovered who were not treated with serum. This reversal of mortality statistics has been the experience throughout the world since the introduction of the anti-meningitis serum. In the New York City epidemic of 1904-5 the mortality was 90 per cent. among 2,000 cases. In 1906 among 1,032 cases reported during the height of the epidemic 812 died—a mortality of 78.7 per cent. In 1907 among 828 reported cases there were 642 deaths—a total mortality of 77.5 per cent. The following tabulation of a few of the reported statistics bears out this remarkable reversal in figures since the introduction of serum treatment:

<table>
<thead>
<tr>
<th>Reported by</th>
<th>Cases Treated with Serum</th>
<th>Cases Treated without Serum, Percentage Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flexner</td>
<td>1,400</td>
<td>31.4</td>
</tr>
<tr>
<td>Steiner</td>
<td>2,280</td>
<td>37.0</td>
</tr>
<tr>
<td>Netter</td>
<td>100</td>
<td>28.0</td>
</tr>
<tr>
<td>Dopter</td>
<td>402</td>
<td>16.44</td>
</tr>
<tr>
<td>Levy</td>
<td>165</td>
<td>18.18</td>
</tr>
<tr>
<td>Sophian</td>
<td>161</td>
<td>15.5</td>
</tr>
</tbody>
</table>

**PROPHYLAXIS OF EPIDEMIC MENINGITIS**

Epidemic meningitis is caused by the meningococcus. The disease primarily begins as an infection of the nasopharynx by the meningococcus. The organism can be demonstrated in the secretion of the nose and throat in 90 per cent. of the stricken during the first 10 days of the illness. During epidemics a large percentage of healthy contacts become infected with the organism and harbor the meningococcus in their noses and throats. The great majority, however, fully 95 per cent., of such contacts—healthy carriers—do not suffer otherwise from the presence of the meningococcus in their noses and throats, except possibly to develop a slight nasopharyngitis. During epidemics as many as 55 per cent. of all healthy individuals exposed to the disease become healthy meningococcus carriers. The organism may remain in the nose of these carriers for a very short time and disappear spontaneously. It may disappear for a short time and then recur at intervals or may persist for a very long time—months or even years.

Healthy meningococcus carriers are the serious menace during epidemics, and are the immediate cause of the spread of epidemics. The carriers propagate the organism, producing other carriers, a small percentage
of whom develop the disease. In addition these carriers are always in constant danger of developing the disease themselves should their resistance be lowered.

Prophylactic measures against epidemic meningitis must, therefore, be concerned with (1) measures of quarantine against carriers both among the ill and healthy so as to prevent the further dissemination of the organism; (2) employment of measures to destroy the organism in the nose and throat of known carriers, and (3) employment of specific measures (such as are used against typhoid fever) to produce immunity among as many healthy individuals as possible in an infected community, of course, preferring individuals who had been exposed to the disease.

**QUARANTINE**

All prophylactic measures, especially quarantine, are really only indicated during epidemics. The presence of sporadic cases alone does not warrant using severe prophylactic measures.

All cases of epidemic meningitis must be strictly quarantined. Quarantine should only be raised when the patient has recovered and when two or more cultures of the nose and throat have confirmed the disappearance of the meningococcus.

The nurse and other attendants of those ill should use the same precaution as in treating other contagious diseases. The sick-room gown should be worn, and where there is close contact with the disease, a gauze face mask. Special care must be taken that the patient does not cough in one's face. All attendants should employ the general prophylactic measures which will be described in the succeeding pages.

All discharges from the nose and throat of the patient must be carefully destroyed. Likewise all excreta, especially the urine, should be thoroughly disinfected and the dressings used in the treatment of complicating infections of the eye, the ear, the secretion of herpes should be immediately destroyed.

All healthy members of a family in which meningitis has occurred should be quarantined on suspicion until a culture of the nose and throat is taken. Positive culture demonstrating the meningococcus indicates close quarantine with the use of local antiseptic measures for the nose and throat. Quarantine should only be raised when the cultures of the nose and throat on two successive occasions prove negative. During severe epidemics close contacts, even though their nose and throat cultures prove negative, should be quarantined arbitrarily for a period of at least a week, during which time they should use antiseptic sprays for the nose and throat.

The measures of strict quarantine controlled by cultural studies are just as practicable and possible in epidemics of meningitis as in epidemics
of diphtheria. That it is feasible and possible has been proved in the control of small institutional outbreaks, and especially well demonstrated in the Texas epidemic of 1912. The writer at that time, with the support of the civil authorities, was able to introduce strict measures of quarantine. Wherever possible all cases of meningitis were removed to a special meningitis hospital. All homes in which meningitis occurred were immediately quarantined. Close contacts were arbitrarily segregated for at least a week, even if cultural studies of the nose and throat proved negative.

A central laboratory was established from which a number of assistants daily went out to the quarantined homes (families in which cases of meningitis occurred). The assistants carried swabs and cultural material for smears of the noses and throats. Fairly accurate reports could be made within 24 hours. A very simple method is to use ordinary throat culture swabs and Loeffler's tubes of culture media. After the tubes are inoculated they are incubated at 37° C. for 18 to 24 hours. Smears are then made from the surface growth and stained with Gram's stain. If Gram-negative diplococci be found a tentative positive report is given, while further cultural studies are made to identify the Gram-negative organism so as to prove whether it be the meningococcus or one of the other members of the Gram-negative group of cocci. The growth is inoculated on several other slants after first carrying through several water blanks. If the meningococcus be present typical discrete colonies usually develop within 18 to 24 hours which can almost be absolutely identified by morphology alone.

During the Dallas epidemic a great many healthy contacts were quarantined in this way. They were informed that they were positive carriers so that they could immediately use prophylactic measures in the form of sprays and prophylactic specific treatment; they thus not only protected themselves in destroying the meningococcus in their nasopharyngeal secretions, but at the same time protected the community. It is true that not all carriers can be isolated during an epidemic. Each positive carrier, however, is a severe menace, and every one who is quarantined and prevented from further spreading the infection helps considerably in stamping out the epidemic.

In Dallas quarantine was controlled in this way in many families. After a period of quarantine lasting about a week cultures of the noses and throats were again taken. If negative on two successive occasions quarantine was lifted.

The community in general was warned of the nature of the infection and advised not to congregate in crowds, to keep the homes properly ventilated and clean, and to guard against promiscuous spitting. Schools were temporarily closed. People were especially warned to be careful to prevent attacks of common cold.

During epidemics cases of multiple infection are much more common than supposed. Strict measures of quarantine immediately with the sim-
ultaneous application of general prophylactic measures undoubtedly help to reduce the number of these multiple infections.

Medicinal treatment in the form of sprays, local applications, and internal medication employed as prophylactic measures are especially indicated among exposed people, and more especially for known healthy carriers. These expedients, however, should also be employed by all members of a community where an epidemic is raging.

Local treatment of the nose and throat of known and unknown carriers should be in the nature of mild, cleansing douches and mild antiseptics. Care should be taken to select an antiseptic that is not irritating. Irritating antiseptics by inflaming the tissues only predispose more to the infection. In the writer’s experience the simple, mild, non-irritating treatment, consisting of mild saline douches, three times a day at 6-hour intervals, followed by spraying with weak peroxid solution (½ to 1 per cent.), is very efficient. Positive carriers after such treatment became negative in a very few days. A number of controls without such treatment, when examined after a week, still harbored the organism, whereas the meningococcus could no longer be found in the secretions of those treated. Other antiseptics may be employed and are useful. Some have recommended argyrol, protargol, chlorin water, menthol, and pyocyanase. The writer found that hydrogen peroxid preceded by salt solution gave the most rapid results. Other observers, however, found that the antimeningitis serum used as a spray gave the quickest results. One of the principal objections to the use of the antimeningitis serum undiluted is that the antimeningitis serum usually marketed contains a strong preservative varying from .2 to .4 per cent. tricresol, which is very irritating to the mucous membrane of the nose and throat.

In the French army regular routine treatment for the nose and throat is used by all members of a garrison in which the disease has occurred. The throat is swabbed regularly with 3 per cent. iodin, followed by gargling with peroxid of hydrogen. In addition an inhalation mixture is recommended. The preparation suggested by Vincent and Bellot (16) follows:

<table>
<thead>
<tr>
<th>Iodin</th>
<th>12 gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guaiacol</td>
<td>2 gm.</td>
</tr>
<tr>
<td>Thymol</td>
<td>35 egm.</td>
</tr>
<tr>
<td>Alcohol, 60 per cent.</td>
<td>200 gm.</td>
</tr>
</tbody>
</table>

This form of treatment is rather rigorous and unnecessary. The milder treatment of saline douche and peroxid spray suffices and is unobjectionable. The severer treatment used by some, and in the French army, is so objectionable that probably most often it is not done carefully and missed by the men so that the purpose is altogether defeated.

Urotropin, on account of its antiseptic properties and its elimination
through the nasal mucosa and through the urine, and its excretion into the cerebrospinal fluid, naturally suggests itself as a suitable prophylactic against the disease, and one that might be generally used among healthy individuals. The writer suggested the use of this drug in the 1912 epidemic in the Southwest. It was employed very extensively. It was very difficult, however, to make observations on the possible efficacy of this drug alone, since in almost every instance where it was used other local measures as sprays and douches were also employed. Flexner, in his experimental work, found that the preliminary administration of the drug in monkeys afforded them some protection later against the injection of poliomyelitis virus experimentally injected. This, too, points to the possible efficacy of the drug.

Specific Prophylactic Measures

The trend of all modern therapy of infectious disease is toward the elaboration of specific measures which will directly influence and counteract the infectious agent. In treating infectious disease sera and antitoxins have been used to neutralize and destroy the infection—well illustrated in the use of diphtheria antitoxin in diphtheria, the therapeutic use of the antimeningitis serum, tetanus antitoxin, streptococcic sera, and other immune sera.

The purpose of vaccines in treating disease is to stimulate the patient to produce immune bodies in larger quantities than he has himself been able to generate. Thus we see the successful therapeutic use of staphylococcus vaccine, acne vaccine, and other vaccines.

Sera and vaccines have likewise been used to prevent disease. The injection of an immune serum into a person exposed to a disease for which the serum is specific will give him immediately a quantity of immune bodies with which to combat the infection. This period of protection, however, only lasts as long as these immune bodies persist in the system. They are usually eliminated within a few weeks—as a rule within 2 to 3 weeks. Diphtheria antitoxin is perhaps the best illustration of an immune serum frequently used to combat disease. Its use among exposed members of families where diphtheria has occurred has prevented in most instances the appearance of multiple infections of diphtheria. Likewise tetanus antitoxin, when used in sufficient doses, affords almost complete protection against tetanus during the period that the antitoxin remains in the system. As a rule this temporary protection of 2 to 3 weeks suffices, since the infectious agent very often lodged in the healthy tissues frequently disappears or dies out during this period of protection. Sometimes, however, it persists in the tissues and may cause disease later.

Permanent protection can be produced by the use of vaccine. The patient is stimulated to produce his own immune bodies which remain in
the system for very long periods, often for years. The advantage of sera over vaccines lies in the fact that the former produce immediate immunity and give the patient protection at once, whereas the latter require at least the period of a week after the first injection before any appreciable immunity occurs. Then, too, immediately after the injection of a vaccine a negative phase may occur, during which period the patient's resistance is lowered so that there is added temporary danger of the disease occurring if the infectious agent be present in the tissues. The dangers of the negative phase can to a very great extent be eliminated by proper precautions, particularly as to dose and in the use of other prophylactic measures that will be described later.

The best known and most successful example of vaccination against disease is the use of typhoid vaccine. Typhoid fever—a dreaded garrison disease—has been almost entirely eliminated in armies where typhoid vaccine has been properly employed. Similarly the use of typhoid vaccine in civil communities and in hospitals has very materially reduced the occurrence of the disease.

The great boon in the establishment of successful specific prophylactic measures against as dangerous a disease as meningitis is apparent. During epidemics work in whole communities is very often paralyzed. The spread of the disease through the medium of healthy carriers, the great uncertainty as to whom the disease will next affect, are sources of great anxiety. Reliable, specific, prophylactic treatment would be most gratefully welcomed by everybody.

A moderate dose of antimenigitis serum injected subcutaneously undoubtedly affords considerable protection against the disease for a few weeks. During the Texas epidemic the writer advocated the widespread use of this expedient, especially in communities where multiple infections were common. Doses of 10 to 15 c. c. were recommended. The measure was used principally among close contacts. No case of secondary infection occurred in those who had been so protected during the period in which protection would be expected; that is, 2 to 3 weeks after the dose. One individual, a porter at the Meningitis Hospital, developed meningitis about 6 weeks after he had been injected. The great objection to the measure is the fact that protection is only afforded for a few weeks after a single dose, and the fact that the injection of so large a dose of unrefined serum is commonly followed by an attack of serum sickness which, to say the least, is extremely annoying. Individuals so injected are also in danger of developing anaphylactic shock should they subsequently require an injection of horse serum whether it be for a subsequent attack of meningitis or for use in other disease, as diphtheria, tetanus, or other infection.

The danger of serum sickness may be eliminated to a marked degree by reducing the dose of the serum. The writer is now inclined to believe that a dose of 5 c. c. of the usual unrefined serum will afford ample protec-
tion against the disease. Even a greater reduction in the dose can be made by using a refined serum so that the relative immune units are still retained. The writer is now making observations on this subject to determine the relative potency of a concentrated serum.

The danger of anaphylaxis is a more important one, especially if the patient should subsequently develop meningitis and require the therapeutic use of serum immediately. In such an event the patient should first be injected with 1 to 2 c. c. of serum subcutaneously. If the patient does not react, or even if he does react, a larger therapeutic dose of serum can be injected within a few hours with less danger of developing anaphylaxis. (See discussion under Serum Sickness.)

The field of prophylactic serum vaccination against meningitis has not been studied sufficiently. Extensive observations will undoubtedly afford very interesting data.

Prophylactic meningococcus vaccination against meningitis naturally seems the most direct method of protecting a community over a long period of time. Clinical and laboratory studies of epidemic meningitis yield data that are favorable to the application of this measure. Epidemic meningitis is a bacterial disease. One attack with recovery affords almost complete protection against the disease. Immune bodies can be readily demonstrated in the blood during the course of the disease. Agglutinins and opsonins have been demonstrated in quite high dilution during the disease and precipitins and complement-fixation bodies have similarly been found, though in smaller quantities. Immune bodies have been demonstrated in the blood of those recovering from epidemic meningitis through the use of the blood serum of recovered cases in treating those acutely ill with epidemic meningitis. In a few cases the blood serum so used by intraspinal injection gave fair results. Similarly all of the above-mentioned immune bodies have been demonstrated in the cerebrospinal fluid of meningitis cases, though, of course, in very small quantities.

Likewise immunity studies on small and large animals have proved that very high immunity can be produced by vaccination with increasing doses of dead and live meningococci. A very simple experiment is the injection of rabbits with killed meningococci. A few doses of vaccine will enable one to protect the rabbit against a larger lethal dose of culture. The use of goats, sheep, monkeys, and horses for the production of a highly immune antimeningitis serum which has been used so successfully in treating the disease in human beings has enabled more accurate and thorough studies of such sera with the demonstration of immune bodies of all orders in very high dilution.

Influenced by these facts, the writer felt justified in advocating the use of prophylactic vaccination during the height of the 1912 Texas epidemic, since the disease was spreading in spite of all measures employed. He recommended doses of 500,000,000, 1,000,000,000, and a third dose.
of 1,000,000,000 at weekly intervals. Relatively very little discomfort, no more than that following typhoid vaccination, was experienced after the injections. Several hundred people were injected within a period of about 6 weeks. Almost all who were vaccinated had been exposed to the disease, many being doctors and nurses who were in constant touch with the sick. None of those who were fully vaccinated with three doses developed the disease. One nurse and a physician contracted meningitis after incomplete vaccination, two doses only having been given. In both instances the disease was mild and recovery prompt. Eleven other nurses who were not vaccinated developed meningitis, the disease being very severe in some instances.

Toward the end of the epidemic the writer was able, with the assistance of Dr. Black, of the Southwestern Medical College, to undertake experimental observations on the effect of vaccination with varying doses of meningococcus vaccine. Eleven medical students volunteered for the study. The students were divided into two squads. The members of one squad were injected with 500,000,000 of killed meningococci as the first dose, and 1,000,000,000 as the second. The others were injected with 1,000,000,000 killed meningococci as the first dose, and 2,000,000,000 as the second. Injections were made at 7-day intervals. Some of the members of the first group received a third injection of 1,000,000,000 killed meningococci, and some members of the second group were injected with 2,000,000,000 killed meningococci. Observations were made on the local and general reaction and on the blood picture.

The vaccine was prepared from a strain of meningococcus which had been isolated from a case of meningitis in Dallas. The vaccine was prepared as follows: The organism was grown on glucose agar from 18 to 24 hours, then washed off in salt solution, shaken thoroughly, standardized, and killed by heat in a water bath at 50° C. for one hour.

A slight leukocytosis occurred in practically all students after the injection, the blood picture returning to the normal on the third to fourth day. There was little change in the total differential blood count. On the whole the blood smear and count showed negligible changes.

**Studies of the Immune Body Content in the Blood of the Vaccinated.**

—About 1/2 c. c. of blood was obtained from the finger of the vaccinated and collected in sterile glass ampoules every 4 days. After clotting, the tube was centrifuged and the serum separated. Suitable dilutions were then made in salt solution and examinations made for the presence of immune bodies, agglutinins, and complement-fixation in the blood.

Agglutinins developed rapidly in all the vaccinated as early as 4 days after the first dose, good agglutination being obtained in dilutions of 1-20 to 1-60. After the second dose most of the sera agglutinated in dilutions of 1-100 to 1-500 a few days after the injection. The examinations a week later where no further injections were given showed an increase in the ag-
glutinating power of the serum up to 1-1000 to 1-500. The greatest response occurred in the students who were injected three times. There was relatively little difference in the degree of agglutination in the sera of those who were injected with the smaller doses as compared with those who were injected with the larger.

Complement-fixation studies showed an increase in the third order of immune bodies in very much the same ratio as in the case of the agglutinins, though the total increase in quantity of these immune bodies was not as high as in the agglutinins. At the end of the third week some of the sera showed fixation in dilutions of 1-200 of the sera, this being a very high degree of fixation. As in the case of the agglutinins, so here there was relatively very little difference in the response as to the formation of immune bodies after the larger doses in the one group as compared with the smaller doses in the other group.

**Clinical Reaction after the Injection of Vaccine.**—The local reaction is very much the same as after injection of other vaccines, notably the injection of typhoid vaccine. A few hours after injection there are redness, swelling, and tenderness at the point of inoculation. Some subjects react much more severely than others. Pain in any marked degree rarely lasts longer than 24 hours. One would expect the later injections to be more painful than the initial. In some instances this is true, but in the writer's experience the later injections, even though they be in greater doses, are followed by much less reaction.

General constitutional symptoms are frequently missing. Most often the patient complains of moderate headache and general malaise. Occasionally there is a rise in temperature of 1° to 3°. Sometimes, however, there is a marked rise in temperature to 104° or even 105° F. The patient may suffer from nausea, have general bodily pain and vomit. Labial herpes develop in some cases.

Sometimes an alarming group of symptoms occurs. About 8 hours after the injection the patient may complain of severe headache, have rigors, vomit, and complain of pain in the nape of the neck. After a few hours the symptoms improve and then entirely disappear within a very short period. These symptoms are particularly alarming on account of the pain referred to the nape of the neck and the suspicious symptoms of meningitis. Even a superficial examination, however, will readily exclude the true disease. All of the other active signs of meningitis are missing. The patient is, as a rule, not acutely ill, improves very rapidly, and has absolutely clear mentality. This symptom-complex is most apt to occur after initial large doses. The condition can probably be explained by the nature of the meningococcus and its effect upon the human being. The probable occurrence of meningitis as a complication of the initial meningococcus sepsis can best be explained by the special affinity of the meningococcus and its toxic product for the meninges. After there have been
sufficient depression and irritation by these toxic products then the meningococcus proper can localize in the meninges and set up the true infection. If this theory be true, one can then explain the occurrence of the peculiar symptoms after the injection of a large dose of meningococccic vaccine. The soluble products of the dead meningococci irritate the meninges the same as do the soluble products of the live organism, though, of course, to a much less degree. This, then, explains the clinical symptoms suggestive of mild meningeal irritation.

As a result of this experience the writer has advocated the use of a smaller initial dose of vaccine, and now recommends an initial dose not over 100,000,000 killed meningococci.

**Analysis of Vaccination Studies.**—A study of the observations on the vaccinated students demonstrated that a vaccine properly prepared and injected in adequate doses stimulates a prompt response in the formation of immune bodies immediately after the vaccination. The group of students who were vaccinated with the smaller doses formed immune bodies in almost as large quantities as those who were injected with the very large doses. The local reaction is very much the same as after other bacterial vaccines. Occasionally a subsequent dose of vaccine will result in the formation of an abscess in which some of the dead meningococci may be found. The general reaction in most instances is also the same as after the use of other vaccines. After the employment of very large initial doses there sometimes occurs a group of symptoms which, while not serious, may be alarming to the inexperienced. The symptoms of suggestive meningitic inflammation subside very promptly—within a few hours. The writer has not been able to demonstrate the occurrence of a negative phase by examination of the blood. It is now recognized that the so-called negative phase is a very much exaggerated condition, provided, of course, that ordinary every-day precautions of using suitable, not excessive, doses are observed. Among the several hundred clinically vaccinated during the Dallas epidemic almost all had been intimately exposed to the disease. No cases of meningitis followed the use of vaccine, even though meningococci could be demonstrated in the nasal secretions of some of the vaccinated. The data of several hundred vaccinated during the Dallas epidemic were, of course, far from conclusive. The fact, however, that many multiple cases were occurring during the epidemic, and that no case occurred among those who had been fully vaccinated, even though many of the vaccinated were most intimately exposed to the disease, must be of some significance. The occurrence of two cases in a physician and a nurse who had been incompletely vaccinated sounds the same warning as did the apparent failure during the first year of typhoid vaccination. One must be most careful to select a vaccine which is potent. If possible, a strain of meningococcus that has been demonstrated to stimulate the production of immune bodies in large quantities should be used. Furthermore, the
vaccine must not be overheated. A temperature of 50° C. suffices to kill the meningococci. Preservatives must not be added in excessive quantities. If good, careful technique be used in the preparation of the vaccine a very minute quantity of preservative (.1 per cent. tricresol) will suffice. The desirability of examining the blood of the vaccinated to actually determine whether or not immune bodies have been produced is apparent.

Encouraged by these observations, the writer determined to study further the effect of vaccination, and to note the duration of immunity after vaccination and to follow the clinical course of as many vaccinated subjects as possible in order to ascertain whether protection was afforded, especially where the vaccinated were intimately exposed to the disease during epidemics. During the following year, 1913, Texas had a moderate amount of meningitis, though it was really free from an epidemic. Vaccine was used in quite a considerable number of people; it was employed both in civil communities and in institutions. The writer had no way of definitely finding out the number of people vaccinated. As far as he could judge there were at least 5,000. He could find no record of meningitis developing among those vaccinated. He was personally able to follow the vaccinations among 300 people in his immediate city. Most, or all, of the vaccinations were in families in which the disease had occurred. In no instance was there a case of multiple infection. Prophylactic vaccine against meningitis was exploited during the year 1913 by a number of manufacturers of biologic products. The measure, therefore, was used in moderate quantities all over the country. In 1913 quite severe epidemics occurred in Tennessee, Arkansas, and Nebraska. Prophylactic vaccination was liberally employed in these communities. As far as can be learned from reports, the vaccinations appeared to be successful.

Undoubtedly the clinical observations must definitely establish the status of this measure. Observations must be made in many thousands of cases before any positive deductions are warranted. The clinical data so far, however, are encouraging.

The writer examined the blood sera of 6 people whom he had personally vaccinated a year and a half previously, and demonstrated by the complement-fixation test immune bodies in all. Two of the vaccinated had been injected with but two doses of vaccine, 100- and 500,000,000 killed meningococci respectively; the others had been injected with 100-, 500-, and 1,000,000,000 killed meningococci at 7-day intervals. In all there was equally good complement fixation of the serum in 1:100 dilution.

Wherever possible, the blood serum of the vaccinated should be examined about a week after the last dose of vaccine. The simplest method is the complement fixation test. The following technique is suggested: Prepare a suitable antigen by growing the meningococcus on glucose agar for 18 to 24 hours. Wash off the growth in salt solution, heat at 50° C. for 2 hours, then allow to autolyse from 12 to 24 hours. It may then be
filtered or used direct. The antigen will usually be potent for a few days. After that, however, it will become anticomplementary. A more stable antigen may be prepared according to the method suggested by McNeil of the New York Research Laboratories. This consists of growing the culture in salt-free agar, washing off the growth in distilled water, and heating at 50° C. for three hours, then immediately filtering through a Berkefeld filter. The clear filtrate is stable for longer periods, from a few weeks to a few months.

It is desirable to make an antigen from a number of different strains, since it has been proved that the meningococcus family, like other organisms, is made up of many strains of the organism. One should, therefore, include in the antigen as many strains as possible. The selection of different strains, however, can only be made by differentiating the strains after examining a great many organisms by laboratory serological methods. This differentiation is rather difficult, and the rough, cruder method of simply selecting a number of different organisms isolated from different cases usually suffices.

The other materials in the test are the same as for any complement-fixation test done according to the Wassermann method. The patient's blood serum should be obtained. A simple method is to collect the blood in a capillary pipette, ¼ to ½ c. c. of serum sufficing. The Wassermann sheep hemolytic system is a convenient method, though the Noguchi method is just as good. In using the Wassermann system the writer, following McNeil's suggestion, has been using one-tenth the bulk of the whole test, using, therefore, in proportion, instead of one-tenth of the patient's serum, one-hundredth; instead of 1 c. c. of corpuscles, .1 c. c.; instead of .1 c. c. of complement, .1 c. c. of a 10 per cent. solution complement, and so on. The technique is simple. The antigen should first be titrated to determine the degree of dilution necessary to eliminate anticomplementary action, and at the same time retain strong binding power, as proved by testing with a known positive serum.

A test of this kind is not concerned with the quantitative findings, but rather with the determination as to whether immune bodies are present. Therefore, the writer has been accustomed to use simply 1-100 and 2-100 c. c. of serum (corresponding to the .1 and .2 for the full Wassermann test). The antigen and complement in suitable quantities should be added and incubated for one half hour, then the corpuscles and the antisheep amboceptor added, and in turn incubated for one hour. Of course negative and positive controls are employed in every test. Readings are then made. Positive reaction is obtained in most instances after full vaccination. Failure to obtain positive vaccination should make one inquire into the preparation of the vaccine used, especially as to the temperature employed in killing the meningococci, and to look for possible idiosyncrasy on the part of the patient.
This technique is essentially the same as is now commonly used for the
diagnosis of gonococccic infection by the complement-fixation test. Any
well-equipped laboratory should be able to do the test at only a moderate
cost to the patient.

The writer has not been able in his subsequent studies to determine any
great danger from the negative phase. He was especially impressed, how-
ever, with the desirability of beginning with an initial small dose, pre-
ferably not over 100,000,000 killed meningococci. It is often quite diffi-
cult to obtain a coincident examination of the secretions of the nose and
throat before vaccination. In about a dozen instances the writer has found
meningococci in these secretions at the time of vaccination. These cases
showed no greater reaction than the others vaccinated. In a few instances
where opportunity was afforded for a subsequent examination of the nasal
secretion—from 1 to 2 weeks after the vaccination—the organism had
apparently disappeared, though no local treatment had been employed.
As an extra precaution, however, it might be well to suggest local treatment
of sprays and nasal douches for the first week of the vaccination period.
Where the vaccinated subject has been very intimately exposed to the
disease it would be well as an added precaution to first take cultures of
the nose and throat. If positive it would be safer to use sprays for the
nose and throat and to take urotropin internally for a few days.

Experience with prophylactic vaccination so far undoubtedly warrants
further study. Observations should be made coincidentally by the clin-
ician and the laboratory worker, and in all instances, if possible, a vaccine
properly prepared should be used. The special precautions in the prep-
paration of a vaccine are the selection of a suitable strain which will stimu-
late the production of immune bodies in the persons vaccinated and care
not to heat the vaccine over 50° C. A minimum amount of preservative
should be used.

In the order of their importance prophylactic measures against epi-
demic meningitis may be summarized as follows:

Quarantine of all sick and as many known healthy carriers as possible.
Arbitrary quarantine should be enforced for a period of at least a week
or 10 days. Wherever possible the period of quarantine should be deter-
mined by cultural examination of the nasopharyngeal secretion, raising
quarantine only when cultures of the nose and throat have been proved
negative for the meningococcus.

The use of mild antiseptic sprays for the nose and throat, one of the
simplest being a spray of 1/2 to 1 per cent. peroxid of hydrogen.

The use of urotropin internally in doses of 25 to 35 grains daily.

Prophylactic meningococccic vaccination. Three doses are desirable,
beginning with a small dose of 100,000,000, the later doses being 500,-
000,000 and 1,000,000,000 of killed meningococci respectively injected
at weekly or 10-day intervals.
During periods of very severe epidemics, where there is very intimate exposure of healthy people to the sick, and where multiple infections of meningitis are occurring, immediate protection may be obtained by means of a small dose of 5 c. c. of the antimeningitis serum injected subcutaneously. The protection afforded by this measure only lasts for a period of about 2 weeks.

REFERENCES


STATISTICAL AND GENERAL REFERENCE

5. Sophian. Epidemic Cerebrospinal Meningitis, St. Louis, 1913.
CHAPTER XXI

GONOCOCCAL INFECTIONS

ERNEST E. IRONS

The prevalence of gonococcal infection in its various forms, the serious and disabling character of the lesions to which it gives rise, and the frequent inadequacy of symptomatic treatment in limiting the disease afford ample justification for the extensive study of methods of the specific treatment of the past ten years.

The treatment of gonococcal infections by serum and vaccines has been the subject of the same exaggerated statements as has been the case in the discussion of specific methods of treatment of other forms of bacterial infection, and in many instances these claims have been so evidently mistaken and unfounded that workers otherwise disposed to grant a hearing to this method of therapy have been led to discard it as unworthy of any consideration.

In the treatment of the complications of gonorrhea, however, there has accumulated a considerable mass of evidence which seems to warrant a critical inquiry into the question of what may be reasonably expected from active and passive immunization. From the standpoint of practical therapy we may ask the following questions:

1. To what extent does immunity develop in gonococcal infections?
2. To what extent can this immunity be measured by experimental methods?
3. Does the inoculation of gonococcal vaccines in man modify immunity to a degree measurable by available methods?
4. If such inoculations can produce a measurable increase in immunity, does this increase influence the course of the infection in man to a degree sufficient to warrant their use as therapeutic agents?
5. Is the use of these specific substances attended with danger to the patient?

In this chapter it is proposed first to review some of the more important points in the symptomatology of gonococcal infection, second, to discuss the reactions of immunity, and, lastly, to inquire into the evidence
for and against the employment of specific methods of treatment in gonococcal infections in man.

CHARACTERISTICS OF METASTATIC GONOCOCCAL LESIONS

Successful diagnosis and specific, as well as general, treatment of gonococcal lesions depend on a clear knowledge of the pathology of the disease. A brief summary of the more important clinical features of general gonococcal infections will assist in the subsequent discussion of the results of treatment by sera and vaccines.

The relatively benign character of the lesions caused by the gonococcus is a matter of general clinical experience. Definite suppuration is rare, considering the frequency of metastatic lesions. The involvement of serous membranes, particularly those of joints, tendon sheaths, burse, and the endocardium and pericardium, is a common observation; periarticular and periosteal inflammation is by no means unusual, and there is scarcely a tissue of the body which has not been found the site of gonococcus infection.

In spite of its reputed benign character as regards suppuration, the gonococcus more than makes up in the chronicity of its lesions what it lacks in virulence, and is able to live for months and even years in some latent focus, and then, often without apparent exciting cause, to initiate a lesion in some distant portion of the body.

The port of entry of the gonococcus is usually the genital tract, though general infection with accompanying arthritis has followed gonococcal ophthalmia. Metastatic lesions have been seen to follow simple anterior urethritis, and even balanitis, without urethritis (Macaigne and Finet), but as a rule the deeper tissues are involved before general infection occurs. Posterior urethritis (Colombini, Ahlman), epididymitis (Bjelogolowsy, Prochaska), periurethral abscess, and prostatitis in men, Bartholinian abscess and pyosalpinx in women are the usual antecedent lesions. Ullman (72), in a discussion of gonococcal septicemia, has called attention to the frequent involvement of the prostate, and in several cases has demonstrated gonococci microscopically and by culture in thrombi from the prostatic and neighboring veins. The local extensions of gonococcal infection, epididymitis in the male, and pyosalpinx in the female, are among the most frequent complications of gonorrhea.

Gonococcal Arthritis.—The clinical picture of gonococcal arthritis is well known. In an ordinary case, following by a few days or weeks an initial urethritis or the lighting up of an old urethral infection, the joint involvement, with pain, tenderness, swelling, and frequent effusion, the occasional involvement sometimes alone, or more often with others, of the sternoclavicular, temporomaxillary, sacroiliac and intervertebral joints, the
persistence of the lesion in the primarily involved joints, and the associated clinical symptoms render the diagnosis obvious. An analysis of a large series of carefully observed cases will demonstrate that gonococcal arthritis is almost always polyarticular. Even in the chronic monarticular hydrosynthesis careful examination of the history will frequently show a multiple initial joint involvement.

Transient arthritis in several joints without pronounced swelling, later persisting in one or more joints, is often seen. So also tender points in the periosteum in the neighborhood of joints may appear during acute exacerbations of general gonococcal infections, and after remaining for a few days slowly disappear.

The pathologic changes in the tissues in which the organism of any given bacteriemia becomes localized will depend obviously on the virulence of the organism, the general resistance of the individual, and the anatomic structure and physiologic relations of the region involved. Serous membranes such as those of the joints are continually exposed to physiologic trauma, and for this reason, among others, an irritative lesion which might pass unnoticed in muscle or subcutaneous tissue quickly produces symptoms in the joint.

The bacterial embolus reaches some portion of the periarticular structure and a focus of infection is formed either in the more superficial portions of the joint or deeper in the capsule, or in the fibrous tissue underlying the synovialis. In the latter case the layers of cells of the synovialis become swollen, and at the point of greatest damage desquamation of the altered cells of the synovial surface occurs. This irritation may cause a rapid increase in the synovial secretion, with resulting distention of the joint. The effusion may be sterile or may contain the infecting organism, if it has passed from the original focus through the damaged tissues of the synovialis. D. J. Davis, in a study of experimental arthritis in rabbits, using cultures of streptococcus pyogenes injected into the ear vein, has found that when the joints are examined soon after the appearance of the arthritis the lesion is frequently extrasynovial; later the joint contains a small amount of glairy fluid, in which a few organisms and leukocytes are present; still later a purulent arthritis is found. The normal motions of the joint and the traction of the muscles tend to increase the tension of the focus and favor its extension into the joint.

If the focus of infection lies at a point more distant from the joint surface there may be no involvement of the synovialis, and clinically we have a more or less extensive periarthritis.

The nature of the infecting organism, whether streptococcus, pneumococcus, or gonococcus, and the virulence of the particular strain relative to the resisting power of the body, determine to a large extent the duration and degree of pathologic changes of the arthritis. Acute arthritis often
heals with complete restoration of normal joint structure and function. Or if the infection has been extensive the joint cartilage may be partially destroyed or secondary connective tissue proliferation occur with resulting adhesions between the joint surfaces.

The joints in the more chronic forms of gonococcal arthritis may exhibit but little tenderness, and the multiple deforming arthritis of the small, as well as the large, joints, with relatively little constitutional disturbance, may resemble to a remarkable degree the symptom-complex known as arthritis deformans. The relation of focal infection to the recurrent and deforming arthritides is well illustrated by the various forms of metastatic gonococcal infection; and the series of joint lesions due to the gonococcus form an instructive field for the study of the different clinical varieties of arthritis which may be caused by one organism. Here we have first the ordinary acute gonococcal arthritis, with frequent occurrence of the organisms in the articular fluid, and with periartritis prominently associated. This acute stage may heal, with or without adhesions or ankylosis, or may pass into a chronic or recurrent stage in which there is often a persistent focus of infection elsewhere in the body. The acute stage may, however, subside almost entirely, and after the lapse of months or years the joint changes again advance, but this time without the symptoms of infection, and result in a pathologic condition of the joint identical with that found in arthritis deformans.

The progressive character of the deforming varieties in one instance may be due to successive reinfections, in another may result from continued mechanical trauma by motion and tension of muscles and tendons on previously damaged joint surfaces.

Bouchard (6), in a discussion of the etiology and pathology of spondylitis deformans of the type described by Marie, advances evidence to show that in many of these cases there has occurred an antecedent gonorrhea. He recites the case of a man of 48, a sufferer from spondylitis deformans, in whom he was able to find no other causal agent except the gonococcus, which was demonstrated in the prostate thirty-seven years after the initial infection. The evidence advanced, while not absolutely conclusive, is still very suggestive.

The view that gonococcal arthritis of the spine may result in bony ankylosis has been combated by several writers. Within the past year I have seen two cases of long-standing gonococcal infection in which the bony intervertebral lesions were clearly evident in the skiagrams. One of these patients was a young man who, at eleven years of age, suffered from a gonococcal urethritis, followed by periurethral abscess, recurring multiple arthritis, and aortic endocarditis. In the succeeding years he had successive recurrences of arthritis, and when seen in his thirtieth year had a rigid spine, an acute iritis, and subacute arthritis of the knee.

A history of preceding trauma obtained in cases of gonococcal arth-
ritis may be misleading. The occurrence of the arthritis and trauma may be sometimes a coincidence, inasmuch as the arthritis is most often found in certain joints, as the knee or ankle, particularly exposed to injury. More often, however, it is probable that an extremely mild gonococcemia is present, and the trauma produces a locus minoris resistentiae, which allows the development of the organisms in the tissues of the joint, with resulting symptoms. Diggelmann has called attention to the tendency of gonococcal arthritis to localize in the joints affected in an antecedent acute articular rheumatism.

Associated with arthritis other lesions, such as periostitis of the os calcis, malleoli, etc., bursitis and tenosynovitis, are frequent, and may give a clue to the diagnosis in obscure cases.

Articular Fluid.—Examination of the articular fluid is of great importance and many times leads to the correct diagnosis in doubtful cases. The fluid is usually pale yellow, somewhat cloudy, and rapidly coagulates on standing. In hydrarthroses of long duration the fluid is often extremely gelatinous, passing through the aspirating needle with great difficulty. A cytologic study of the centrifuged fluid will show polymorphonuclear leukocytes in considerable number, with large endothelial cells and very few mononuclear leukocytes. The excess of polymorphonuclear cells may be of assistance in certain instances in which the diagnosis lies between gonococcus and tuberculous arthritis. In the early cases it is often possible to demonstrate gonococci, both in the leukocytes and lying free in the fluid, but the difficulties of the search become progressively greater with the increase in duration of the arthritis.

The proportion of cases in which the gonococcus can be obtained in cultures from the articular fluid is variously estimated at from 30 per cent. to 70 per cent. The more acute cases give the larger proportion of positive results. Cultural methods will often succeed where microscopic examination has failed to show the gonococcus in articular fluids.

The constitutional symptoms of fever and malaise in gonococcal arthritis vary greatly in degree from a slight indisposition with temperature of 99° to 100° F. to the most severe forms of septic or typhoid-like temperature, with delirium, dry tongue, and rapid pulse. Moreover, the severity of the constitutional symptoms is by no means always proportional to the degree of joint involvement. This observation is easily accounted for when we consider that the arthritis may be merely an incident in the course of a general gonococcemia, and that the symptoms are those of general sepsis. Such a case was observed in a young man, the subject of a moderately severe arthritis. Following a few hours of indisposition the fever, which had previously ranged from 99° to 100° F., suddenly rose with a chill to 103° F., and an extension of the arthritis to the right ankle occurred. Within twelve hours after the rise in temperature blood cultures were made, from which the gonococcus was isolated in pure cul-
ture. The organism was isolated also from the effusion in the ankle joint. In the course of a few days the fever subsided, the arthritis improved, and the patient left the hospital in one month, able to return to work. There was no evidence of heart lesion during the illness or convalescence. This case, and others (Oro, Unger), support the contention that the arthritis is but one manifestation of the bacteriemia which is present in the arthritic cases.

Severe toxic symptoms, with high fever, etc., associated with arthritis of the knee, for instance, do not necessarily indicate that suppuration in the joint has occurred. A case illustrating this point was seen in a man with arthritis of the knee, from the effusion of which the gonococcus had been isolated. The fever began to rise, and within a few days he became profoundly ill. There was no other demonstrable focus of infection, and the surgeon in charge, suspecting purulent arthritis, opened the joint. No pus was found. The fever continued and the joint was again opened, with similar result. After five weeks the fever subsided and the patient made a good recovery without demonstrable heart lesion. The gonococcus was not isolated from the blood, but there can be little doubt that in this case the general symptoms were due to gonococcemia.

Iritis.—Metastatic lesions of the eye are occasionally seen in gonococcal infections, and from recent studies appear to be somewhat more frequent than was previously thought. They often are accompanied or preceded by arthritis (in 90 per cent., according to some older statistics), but in some cases the arthritis may be remote. Recurrences of iritis are frequent, and may be associated with new arthritic lesions, or may appear independently of them. The association of a primary iritis with arthritis is a strong argument for the view that the iritis is set up by a localization of the gonococcus in the ocular tissues, rather than by the action of toxins. There is very little evidence in favor of the toxic as opposed to the metastatic origin of gonococcal arthritis, and in the primary iritis occurring in the course of gonococcal arthritis the reasonable conclusion is that it also is metastatic. (Axenfeld, 2; De Schweinitz, 60.)

The gonococcus has been isolated from the deposits in the aqueous humor in some cases. In other cases which have been carefully studied the organisms have not been found in the aqueous, the deposits in which were apparently secondary to the primary lesion in the uveal tract. Such a condition presents a close analogy to the phenomena seen in arthritis, in which the lesion may be (1) periarticular, (2) confined to the subserosa of the joint with a sterile exudate within the joint, or (3) may extend into the joint, in which case the exudate contains many organisms together with leukocytes.

The recurrences of iritis present a somewhat more complex problem, however. It is of course conceivable that in some cases they may be due to renewed localizations of gonococci, particularly in those instances in
which there is a coincident new or recurrent arthritis. The more recent advances in our knowledge of allergy suggest a possible explanation of the recurrent attacks of iritis without constitutional or other evidence of metastases elsewhere, on the basis of a reaction of the sensitized tissues of the eye to a sudden inoculation of gonococcal protein from some hidden and silent focus in the body. There is a very close resemblance between the changes in the eye in recurrent iritis and the reaction seen in a tuberculous iritis following the inoculation of tuberculin. Some observers have noted reactions in gonococcal iritis following the inoculation of gonococcal vaccine, which resembled the reactions following tuberculin. Others have failed to find such a reaction, but the hypothesis is attractive and deserves further study. Positive complement-fixation reactions for the gonococcus have been obtained in such cases.

Gonococcemia.—The occurrence of the gonococcus in the circulating blood in certain forms of gonococcal infection has long been recognized. The demonstration of viable gonococci in the effusions of gonococcal arthritis led to the conclusion that at some time previous to the arthritis gonococci must have been present in the blood. Hewes (25) in 1894 first isolated the gonococcus from the blood in a case of arthritis. The clinical observation that endocardial lesions not infrequently followed local infection by the gonococcus was supplemented by the finding of the organisms in the endocardial vegetations in fatal cases of ulcerative gonococcal endocarditis. Thayer and Blumer (65) in 1896 isolated the gonococcus in pure culture from the blood during life in a case of ulcerative endocarditis, and post mortem demonstrated the organism in the endocardial vegetations. Faure-Beaulieu (16) in 1906 collected 34 cases from the literature in which the gonococcus was isolated from the blood during life. This series included cases of arthritis, endocarditis, and sepsis without local manifestations. Külbä (35), in a review of the literature of gonococcal endocarditis up to 1907, found records of about 100 cases, in 30 of which the organism was demonstrated post mortem either microscopically or by culture.

Cases of continued fever in some instances resembling typhoid have frequently been observed in the wake of gonorrhea, and gonococcal sepsis without demonstrable localization has been recognized as a clinical entity. Thayer (64) in 1905 reported a non-fatal case of this kind in which the gonococcus was isolated from the blood. Dieulafoy (13) has reported a case of gonococcal sepsis with recovery without demonstrable heart lesion in which the gonococcus was isolated repeatedly from the blood.

Hewes (25), Unger (73), Oro (47), and others have isolated the gonococcus from the blood during the course of gonococcal arthritis. Irons (29) in 1909 reported six cases of gonococcal infection with arthritis in which the gonococcus was isolated from the blood. In two of these there was clear evidence of a progressive ulcerative valvular lesion. The
literature of the past four years contains an increasing number of reports of similar cases.

When we consider the prevalence of gonococcal infection, with its attendant complicating involvement of the serous membranes, prominent among which are those of the heart and joints, it becomes evident that the gonococcus is a relatively frequent invader of the circulating blood.

Gonococcal Endocarditis.—In addition to the many instances of ulcerative endocarditis of gonococcal origin there is much clinical evidence to indicate that gonococcal infections of the heart valves may heal, with resulting valvular deformities such as are found in the so-called benign endocarditis following articular rheumatism. The appearance of a murmur in a previously normal heart, together with cardiac irritability without other apparent cause, developing during the course of an infection by the gonococcus, and the occurrence of the signs of valvular defects following gonorrhea, in previously normal subjects are points in favor of this view. Withington (78) has recorded a non-fatal case in which the signs of progressive valvular defect and cardiac irritability developed in the course of a gonococcal infection in a previously normal individual, from whose blood the gonococcus was isolated. The evidence presented in this, and in similar cases cited by Silvestrini, Loeb, Prochaska, and others, argues strongly for the healing of gonococcal endocarditis. In a patient with a gonococcal infection in whom the signs of valvular lesion appear and then subside, without the development of the signs of ulcerative endocarditis, one should be cautious, however, in assuming that the lesion is of the so-called benign type, for the gonococcus, true to its characteristics elsewhere in the body, may persist in the cardiac vegetations and months later give rise to a frankly ulcerative process. Then, too, there is always the possibility of a reinfection of the damaged heart valves, either by gonococci which enter the blood from some persistent focus elsewhere in the body or by other secondary pyogenic organisms.

There are approximately 120 cases of gonococcal ulcerative endocarditis on record, in several of which pericarditis has been present. The clinical picture of the gonococcal form does not differ essentially from forms due to streptococcus and pneumococcus, except possibly in the frequency of lesions in the aortic valves. Külbs, in his analysis of 49 well-authenticated cases in 37 men and 12 women, found the following proportion of valvular involvement: aortic, 28 times; mitral, 8; pulmonic, 6; tricuspid, 1; aortic and mitral, 3; mitral and tricuspid, 1; aortic, tricuspid, and mitral, 1; all valves, 1. The course may be rapidly fatal, or may extend over months or years. In type also, gonococcal endocarditis resembles the other forms in that in some cases the mechanical valvular defect dominates the picture, in others the febrile or septic features are the most prominent. Infarcts rarely become suppurative. Petechiae are apparently less frequently observed than in other forms.
They were present in large numbers in one of Thayer's cases, but are not mentioned in a number of cases in which the complete histories are given.

**Gonococcal Septicemia.**—The term "gonococcal septicemia" is here used in a clinical sense to include the group of cases in which the symptoms of sepsis predominate without pronounced arthritis or demonstrable endocarditis. In endocarditis, sepsis, and arthritis, gonococcemia transient or persistent, is present, and the type of the disease is determined by the lesion which dominates the clinical picture. Faure-Beaulieu, in his review of 34 cases in which the gonococcus was isolated from the blood, found only 3 in which there was no localization in the joints or heart valves. To these may be added one of the cases of Dieulafoy already referred to. These figures, however, by no means represent the relative frequency of the septic type of gonococcal infection.

Padula and others have described a type of remittent fever in the subjects of uncomplicated gonorrhea, with enlarged spleen and other signs of mild general infection. These cases are not unusual in general practice, but the illness is too frequently ascribed to the local lesion or to some assumed but undiscovered focus, rather than to the gonococcosis which a careful blood examination may demonstrate.

Other cases of relatively mild type of septicemia are observed in patients who in months or years past have suffered from an arthritis which has healed or become quiescent. A young man presented the following suggestive sequence of events: gonorrhea 10 years ago; recurrence 8 years ago, with epididymitis and hydrocele; polyarthritis with effusion in the right knee 6 years ago; recurrence of gonorrhea 5 years ago, with no urethritis for 4 years; frequent recurrence of arthritis of knee, which at present is tender, with slight effusion; for five months attacks of irregular fever at intervals of ten days, with occasional chills and general malaise. On admission to the hospital during one of these accessions of fever of five days' duration the patient presented several points of difficulty in diagnosis. The continued fever during the early days of observation, together with the presence of several hyperemic maculopapules on the abdomen, suggested a possible typhoid complicating a previously existing arthritis. The subsequent change in fever curve to that of an irregular type, the absence of later typhoid phenomena, the leukocytosis, the failure of the agglutination reaction, together with the absence of typhoid bacilli and the presence of gonococci in the blood, determined the diagnosis of gonococcal sepsis. On several occasions a mild arthritis of the smaller joints of the hands occurred. The fever subsided during the two months of hospital observation, but at the time of the patient's discharge there was still an evening rise to 100° F. The general physical condition on leaving the hospital was fairly good, and there was no definite evidence of endocardial lesion. Twenty months later the patient reported that he had had frequent attacks of irregular fever, and that he
was now suffering from palpitation and dyspnea. The slow ravages of chronic gonococcal infection, with gonococccemia of probably over two years' duration, and a possible chronic ulcerative endocarditis, are strikingly illustrated in this case.

Still other cases of gonococcal sepsis run a severe and rapidly fatal course, with high fever and pulse, and delirium. A man addicted to the use of alcohol entered the hospital suffering from pain in many joints of two weeks' duration, fever, and mild delirium. On admission urethritis was not observed, and a provisional diagnosis of acute articular rheumatism with alcoholism was made. The discovery of a slight urethritis, followed by detection of the gonococcus in the blood, made the diagnosis clear. The fever curve was sustained without remissions and gradually rose toward death, which occurred on the twentieth day of the disease. Examination of the heart during life gave no evidence of endocardial lesion. A number of small, slightly raised, reddish maculopapules, which partially faded on pressure, were seen over the abdomen. These resembled petechiae somewhat more closely than the roseola of typhoid.

A similar case was that of a woman in whom a provisional diagnosis of articular rheumatism was made. She entered the hospital on the fifth day of her illness, suffering from multiple arthritis involving the ankles, knees, and wrists. A purulent arthritis and periartthritis of a finger joint developed, in the pus of which the gonococcus was found in large numbers. The gonococcus was isolated in pure culture from the pus and from the blood. The fever was of the continued type, without remissions, with signs of profound sepsis, and death occurred thirteen days after the first symptoms were noted.

More puzzling at times are the cases in which the clinical picture closely simulates that of typhoid fever. Dieulafoy (1909) recites the case of a man of 23 who had been sick for eight days, with headache, high fever, and diarrhea. On the abdomen were a number of red hyperemic papules resembling rose spots, the spleen was enlarged, and the patient presented a typical typhoid appearance. Sweating was profuse. Agglutination tests with various strains of typhoid and paratyphoid bacilli were repeatedly negative. The gonococcus was isolated from the blood on several occasions, but no typhoid bacilli were found. The gonococcus was found in the sputum also later in the illness. The fever curve was fairly typical of typhoid with defervescence on the fortieth day. Curiously enough, a few days later the patient suffered a second rise in temperature, which ran a typhoid-like course, and now typhoid bacilli were isolated from the blood, and characteristic agglutination phenomena obtained with the serum.

Dieulafoy reports another case of arthritis with demonstrated gonococccemia in which the fever curve was more irregular. In this instance also typhoid fever followed the gonococccemia.
CHARACTERISTICS OF METASTATIC LESIONS

Malaria and pyemia, due to other pus organisms, may be suggested by the marked oscillations of the fever curve in some cases of gonococccemia. In one of Wynn's cases (70) abscesses over the right hip and in the calves of both legs were observed, in the pus from which gonococci in pure culture were obtained. The gonococcus was isolated from the blood before death. At autopsy, in addition to the multiple abscesses, recent endocardial vegetations were found without definite ulceration. In a second case Wynn observed multiple abscesses over the body, associated with gonococccemia.

Other Complications of Gonococccemia.—Pericardium.—Pericarditis has been noted in a number of cases (usually fatal) of gonococcal septicemia. It is often exudative. Tyree (71), in a review of the postmortem reports in cases of gonococcal infection, finds the pericardium involved in 40 per cent., the lesions varying from slight ecchymoses to serous, fibrinous, hemorrhagic, or purulent exudates.

Lungs and Pleura.—Bressel (7) has reported a case of gonococcal pneumonia, beginning on the fifteenth day of a urethritis. Definite signs of consolidation were present in the lung, and gonococci were found in the expectoration, as well as in the blood. Convalescence began on the eighth day of the pneumonia. Dieulafoy saw in his second case bilateral bronchopneumonia in which the gonococci were isolated from the sputum. Although gonococcal pneumonia may occur, by far the larger proportion of pulmonary lesions is due to infarction, or to passive congestion from the increasing valvular defects of the endocarditis with which they are usually associated.

Pleurisy, often with effusion, is more frequent than pneumonia, and in several instances the gonococcus has been isolated from the cloudy exudate. Prochaska describes a case in which the gonococcus was isolated from the blood and from the pleural effusion. Empyema may develop.

Signs of fluid were made out in one of my series, but may have been due to failing heart action. Two days before death this patient suffered severe pain in the chest, with cough and bloody expectoration, in all probability marking the occurrence of infarction.

Kidneys.—Albuminuria is often remarked in patients suffering from gonococcal infections. Excluding the many cases in which the albuminuria results from an extension of the infection through the genito-urinary tract, there still remain a number of instances in which albuminuria, and at times casts, have been noted. Renal infarction and passive congestion are often seen in the endocarditic cases. Acute renal insufficiency with anasarca has been observed (Thayer), and, post mortem, cloudy swelling with beginning interstitial changes have been found (Harris and Johnston, Wynn). Ahman noted a persistent albuminuria in a patient convalescent from gonococccemia, and there is considerable evidence in favor
of the view that gonococcal infection may give rise to both acute and chronic forms of nephritis.

Spleen.—Splenic enlargement is frequent in gonococcemia either as an acute splenitis such as is seen in other acute infections, or as the result of infarction. In gonococcemia of the typhoid type the occurrence of splenic tumor should be borne in mind. In 42 cases of demonstrated gonococcemia, including arthritic cases, the spleen is reported enlarged in 15. In a number no record as to this finding appears, and in 4 absence of enlargement was noted.

Skin.—The skin lesions of gonococcemia are of decided interest. The influence of various medicaments, such as the balsams which are held responsible for many of the rashes seen in acute genital infections, can usually be excluded in the cases with general infection. Diffuse erythema, erythema nodosum (Prochaska), erythema multiforme (Barbiani), and extensive purpura (Silvestrini, Achar) have been observed. Hyperkeratosis gonorrhoeica has been described, and may be found associated with arthritis. Petechiae are occasionally seen in gonococcal endocarditis, though they are apparently less frequent that in cases due to other pyogenic organisms. A generalized petechial eruption is recorded by Thayer.

A hyperemic maculopapular rash resembling very closely the rose spots of typhoid has been observed in several cases. It was present in two of Thayer's cases, in one of Dieulafoy's, and in two of the series I reported in 1909. Since that time I have seen four other instances in generalized gonococcal infection, with multiple arthritis. Herpes labialis has been observed occasionally in general gonococcal infections. Profuse sweating has been repeatedly noted, especially in the severe cases of endocarditis and sepsis.

Nervous System.—Organic lesions of the central nervous system have been occasionally observed. Prochaska reports a case of purulent cerebrospinal meningitis in which the gonococcus was recovered in pure culture during life from the blood, and post mortem from the purulent meningeal exudate, and from pus in one of the seminal vesicles. Thrombosis of the periprostatic veins was also found, but no trace of infection remained in the urethra. There was no lesion found in the heart or lungs. Fürbinger (22) also has reported a fatal case of cerebrospinal meningitis following acute gonorrhea. Cerebral embolism has occurred in gonococcal endocarditis. Delirium and stupor are often observed in the severe toxemias of gonococcal infection. Multiple neuritis and other affections of the peripheral nerves have been described among the complications of gonococcal infections, but their exact etiology, whether toxic or due to actual invasion of the nerve sheaths by the gonococcus, has not been determined in cases in which gonococcemia has been culturally proven.

Blood.—In addition to the presence of the gonococcus either as a
transient, or oftener as a more or less persistent invader, the blood presents several features of interest in the study of gonococcemia. Polymorphonuclear leukocytosis is almost always pronounced. The relation of the eosinophilic leukocytes described in gonorrheal pus to the leukocytic formula of the blood has been widely discussed. The general opinion seems to be that in the case of the blood at least eosinophilic determinations have no value in diagnosis or prognosis.

Anemia is often a prominent symptom in general gonococcal infection, and gonococcal endocarditis in common with that due to other organisms may present a rapidly progressive diminution in erythrocytes and hemoglobin. For the immunological changes in the blood see page 600.

Liver.—The liver is but rarely involved directly in gonococcemia. Silvestrini (quoted by Faure-Beaulieu) saw an intense icterus in a case of gonococcal infection, in which he isolated the gonococcus from the blood. The icterus appeared on the eighth day of the general infection and persisted for several days. Passive congestion, cloudy swelling, and fatty changes are the most frequent post-mortem findings. Icterus neonatorum has been held to have a favorable influence on ophthalmia (Löhlein).

Other Lesions.—Gonococcal lesions of the cellular tissue, tendons, and muscles as a rule do not cause suppuration, but multiple abscesses, both subcutaneous and deep, have been described (Wynn) in which the gonococcus was the only organism present.

Peritonitis, which usually develops by local extension from adjacent structures in the pelvis, may occur as part of a general gonococcemia. Dieulafoy refers to a case studied by Scherrer, in which purulent peritonitis was associated with a pleural effusion. Previous to his illness the patient, a soldier, had suffered from a mild gonorrhea of a few days' duration. On his return from a march he was seized with chills, high fever, and headache, followed by diarrhea and moderate dyspnea. The tentative diagnosis of typhoid fever was rendered more probable by the occurrence of sudden abdominal distention, tenderness on pressure, and hiccough. On the day following the appearance of the signs of peritonitis the dyspnea became more pronounced and pleural effusion was detected. The patient died on the eighth day. At autopsy purulent peritonitis, pleurisy with effusion, and recent mitral endocarditis were found, and the gonococcus was isolated from the exudates and from the valvular vegetations. There was no intestinal perforation or other visible evidence of typhoid fever.

In severe gonococcemia emaciation is often a striking feature, due no doubt to the continued toxemia and the coincident anorexia.

Diarrhea has been noted at the onset of several cases of gonococcemia. Glycosuria was present in one of my cases of gonococcemia.
DIAGNOSIS

Generalized gonococcal infection in its several clinical forms, such as the arthritic, endocarditic, or septicemic, in which a history of preceding genital infection is obtained, may present no special difficulty in diagnosis. It often happens, however, that gonococcal infection is denied, and on examination no signs of urethritis are found. The disappearance of urethritis coincidently with the onset of chill, fever, and other symptoms of general infection has been frequently remarked by clinicians, and is a fact which should be always borne in mind in otherwise suspicious cases. In the same connection alcoholic excesses have frequently preceded the onset of general gonococcal infection in individuals supposedly cured of an antecedent gonorrhea.

Gonococcal arthritis may simulate very closely acute articular rheumatism. The arthritis may subside rapidly in the joints first involved, thus suggesting the migratory arthritis of rheumatism, the resemblance to which may be further increased by the high irregular fever, occasional mild angina, leukocytosis, and absence of signs of urethritis. From a therapeutic standpoint the amelioration of symptoms by the salicylates will not always serve to exclude gonococcal arthritis, for in certain instances the analgesic properties of these drugs are pronounced.

In the chronic cases still greater difficulties may be encountered. Monarticular gonococcal arthritis may resemble tuberculous arthritis. The absence of demonstrable tuberculous foci elsewhere, the history of an old gonorrhea, and of previous, perhaps transitory, arthritis or periositis, the leukocytosis, and the microscopic and cultural examination of the frequent effusions in the joint, and the specific serum reactions will assist in the diagnosis. Gonococcal inflammations of the articulations and interspinous ligaments of the vertebrae may be extremely chronic and suggest a persistent lumbago. Exostoses, particularly those of the os calcis, are frequently seen following gonococcal infection. Baer (3) has demonstrated the gonococcus in these lesions. The X-ray is of service in determining the location and extent of the bony changes.

In the obscure acute cases of gonococcal arthritis bacteriologic examinations of the exudates are of great value, and blood cultures will at times assist in diagnosis by demonstrating the associated gonococcemia. Some information is to be gained by the reaction which follows the subcutaneous injection of a suspension of dead gonococci. A rise in temperature, increase in leukocytosis, and increase in pain and tenderness in the affected joints follow the injection in some cases.

Ulcerative endocarditis due to the gonococcus presents no constant features which will distinguish this form from cases due to other organisms. Other foci of gonococcal infection and a preceding or coincident arthritis
are suggestive, and often serve to render the diagnosis practically certain. Careful, and, if necessary, repeated, blood cultures, however, are of the greatest importance in demonstrating the gonococcal nature of the endocarditis.

Gonococcal septicemia presents more difficulties in diagnosis than the other forms of gonococcemia. Especially is this true of the typhoid type, in which the fever may be continued with only slight remissions, and the patient may present the picture of severe toxemia with marked prostration, mental stupor, and delirium. Enlargement of the spleen, a rash resembling rose spots, and the diazo reaction of Ehrlich may add to the difficulty of early diagnosis. The presence of leukocytosis, which is practically constant in gonococcal septicemia, the absence of the agglutination reaction, the complications of arthritis and pleurisy, and the occasional irregularities in the fever curve will argue against typhoid fever. Herpes labialis is more frequent in gonococcal infections than in typhoid fever. Finally cultures will demonstrate the presence of the gonococcus and the absence of the typhoid bacillus in the circulating blood.

**THE GONOCOCUS**

**Methods of Cultivation of the Gonococcus.**—The difficulty in growing the gonococcus in the laboratory has been remarked by all who have undertaken a serious study of it. Strains of the gonococcus apparently vigorous suddenly die out after two or three generations, despite all precautions. Other cultures may be maintained for long periods of time by repeated transfer on suitable media. Laitinen (37) recovered the gonococcus from a fluid culture sixty-one days old. The writer has obtained growths from cultures on ascites agar two months old. A number of strains of the gonococcus have been preserved in laboratories over periods of several years by careful transfers on proper media.

There is some difference of opinion as to the virulence of older cultures. Some strains undoubtedly lose their virulence, but others have retained their virulence to a remarkable degree. The age of cultures is an important factor in determining their availability for use in the preparation of vaccines.

The cultural requirements of the gonococcus are well known, but a brief reference to them may be of assistance to those less familiar with the organism. Briefly they are proper conditions of (1) culture media, (2) temperature, and (3) moisture.

**Culture Media.**—The gonococcus grows best on the more highly albuminous media. Many methods for making special media have been formulated, and warmly advocated by those who have devised them. The addition of serum or blood from various animals such as the sheep, ox,
horse, rabbit, pigeon has been suggested, and has proved useful in the hands of different workers.

Perhaps the most satisfactory and least criticized medium is ordinary nutrient agar to which has been added ascites fluid or a few drops of human blood. The latter is always available.

If a patient with ascites (preferably associated with cirrhosis of the liver) is available the ascites is drawn directly into small flasks or bottles under aseptic precautions, taken to the laboratory, and at once heated at 56°-60° C. for one to two hours, and then stored in the ice-chest. Heating for 24 hours at 60° C. does not interfere with the usefulness of the fluid, though a slight precipitate may form.

With care in obtaining the fluid contaminations may usually be avoided, but are readily detectable with proper controls.

The ascites fluid is added to melted tubes of agar after cooling them to 50° C. One part of ascites fluid may be added to two parts of two per cent. nutrient agar, or, if three per cent. agar is used, a somewhat larger proportion of ascites fluid may be used. If, after slanting and cooling, the medium is not sufficiently rigid a smaller proportion of ascites must be used. Ascites fluids are not uniform in furnishing equally favorable media for the growth of the gonococcus, and occasionally one lot of fluid may have to be discarded as unsuitable. This, however, is rare.

Hydrocele fluid, if obtainable, may be used. For fluid media one part of ascites to two parts of bouillon is a convenient mixture.

Blood agar is made by adding to tubes of melted agar properly cooled a few drops of defibrinated blood by means of a sterile glass pipette, after which the tubes either may be slanted or poured into Petri dishes. Media prepared with ascites fluid or blood should be incubated for one or two days, or, if they are to be used at once, suitable controls should be reserved.

The reaction of the medium in which the gonococcus is grown is of some moment. The reaction of the nutrient agar or bouillon base should not exceed 10 acid to phenolphthalein, and some workers have found a neutral reaction to phenolphthalein preferable.

Media have been devised for the cultivation of the gonococcus, which do not contain fluids from human sources.

For many years it has been recognized that some strains of gonococcus are able to grow on ordinary nutrient agar, though as a rule this growth occurs only after some period of cultivation on agar containing serum, and is not so luxuriant as on the latter medium.

Thalman recommended a specially prepared beef agar medium adjusted to 10 acid to phenolphthalein, and was able to isolate the gonococcus from purulent secretions on this medium. A number of other investigators were not able to obtain so good results with Thalman's agar, and
preferred to utilize the more favorable media containing uncoagulated animal or human serum.

In experimental work on carefully controlled cultures Thalman's or similar preparations of agar may at times be preferable for certain purposes to those containing uncoagulated serum, but for the isolation of the gonococcus from human sources blood-agar or ascites agar can be depended on to give somewhat surer results.

Various substances, such as dextrose, maltose, glycerin, nutrose, have been added to media in the hope of favoring the growth of the gonococcus, and in some cases may be desirable, but opinions differ widely as to the advantage to be gained by these additions. As a safe routine procedure the use of media containing human blood or ascites fluid is recommended for the isolation of the gonococcus.

These media are usually available and give a larger proportion of positive results than other modifications. After a few transfers on artificial media the gonococcus often grows more readily and less care as to frequent transfers is necessary. Some strains after a long sojourn in the laboratory may grow on ordinary plain agar, but other strains may fail to acquire this power even after months.

Temperature.—The gonococcus grows best at 35° to 37° C. A rise in temperature of the thermostat to 41° C. or 42° C. may be sufficient to kill the cultures, and a temperature of 39° C. or 40° C. may interfere with growth. In case the room in which the thermostat is placed is subject to wide variations in temperature it is often safer to set the regulator of the thermostat for 35° C. This will allow for unavoidable rises in temperature, and does not seem to be too low for the growth of the cultures.

Care should be taken to avoid chilling of fluids and cultures containing the gonococci in their transfer from the patient to the thermostat. Several workers have called attention to the susceptibility of gonococci in body fluids to slight lowering of the temperature. It is possible that this lethal action is due, not only to the chilling of the organisms, but also to the coincident changes in the fluids themselves, by which antibacterial substances may be set free to act on the organisms. In general, the earlier specimens suspected of containing gonococcus are examined after the removal from the patient the more likely is the organism to be recovered.

Lumière and Chevrotier (41) have recently reported experiments with strains of the gonococcus 12 to 15 generations after isolation from acute and chronic urethritis, in which they were able to recover the organisms from cultures after exposure for 48 hours to temperatures of —17° C. to —20° C.; another series of cultures remained viable after 10 days' exposure to —20° C.

Wassermann reported also the isolation of the gonococcus from the
heart valves of a specimen which had been kept for 24 hours in the ice-box.

Moisture.—Of scarcely less importance than proper media and temperature for the growth of the gonococcus are favorable conditions of moisture. The gonococcus is extremely sensitive to the effects of the loss of moisture, and a freshly prepared medium with moist surface is requisite to good growth.

Plate cultures should be kept in a moist chamber, and test tubes should have snugly fitting stoppers. For the maintenance of stock cultures the use of cork stoppers previously boiled in paraffin, inserted on top of the cotton stopper after careful flaming, is of value in preventing evaporation. A further explanation of the superiority of closely stoppered tubes over those more loosely plugged with cotton for cultivation of the gonococcus is afforded by the studies of Wherry and Oliver. They found that in cultures made by inoculating pus-containing gonococci, very few colonies appeared on the tubes or plates kept under aerobic conditions, whereas many colonies grew in the cultures placed under conditions of partial oxygen tension. The gonococcus therefore appears to be a micro-aerophile, or partial tension organism. Wherry and Oliver (77) employed a modification of the method of Nowak for producing conditions of partial oxygen tension, attaching by rubber tubing, to the tube inoculated with the gonococcus, a tube freshly inoculated with B. subtilis.

Examination of Blood, Exudates, and Secretions for the Presence of the Gonococcus.—Blood Cultures.—The gonococcus has been isolated from the blood in a number of cases.

In obscure cases of sepsis, in ulcerative endocarditis, and in certain acute arthritis blood culture may aid in demonstrating the gonococcal etiology of the infection. The gonococcus may be isolated from the blood by means of fluid cultures, or by ascites-blood-agar plates. Flasks or large tubes containing 30 to 100 c. c. of ascites broth (30 to 50 per cent. ascites fluid) receive 1 to 2 c. c. of blood from the patient.

It is well to inoculate several tubes in this way. Colonies may appear in 48 hours, though occasionally three or four days are required before the small, round, whitish colonies become visible in the otherwise clear medium.

In making ascites-blood-agar plates ascites fluid is added to melted agar after cooling, to which is added one half to one c. c. of blood from the patient, and the tubes at once poured into plates. A number of plates should be made, including controls, because the gonococcus is likely to be present only in small numbers in the blood. Repeated cultures may be required, as the gonococcemia is often intermittent.

Colonies of the gonococcus on blood agar plates are seen after 48
hours, or sometimes only after three or four days, as small, grayish points one to two millimeters in diameter.

Articular Fluids.—Fluid freshly aspirated from effusions in joints is examined in the same way as the blood. Inoculations on blood-agar or ascites-agar slants or plates are made with a few drops each of the fluid. Film preparations of the fluid should be made for direct examination. Cultures may reveal the presence of the gonococcus when no organism can be positively identified as gonococcus in the films.

In gonococcal infections of joints the gonococcus may be isolated in a large proportion of cases if the examination is properly made soon after the onset of the arthritis. Thus Rindfleisch and Nasse obtained the gonococcus in culture from 19 out of 30 cases, Bauer in 19 out of 27 cases. (J. Koch, 34.) Cultures made over one week after the onset of the effusion are likely to show no growth. The gonococcus is usually found alone, but occasionally other organisms such as the staphylococcus are also present. This has been a rare occurrence in the experience of the writer.

Secretions.—For the examination of secretions such as those from the vagina, urethra, or prostate blood or ascites-agar plates are most serviceable. Blood-agar or ascites-agar slants may be used, and are often more convenient and available. Care must be taken not to use too much of the secretion for the inoculation, otherwise the colonies of gonococcus, if present, may be overgrown by other more vigorous organisms. In examining film preparations from secretions care should be taken to distinguish the gonococcus from the Gram-negative coccoid forms of the colon bacillus. These latter forms may be intracellular at times, but the occurrence of the bacillary type, together with the coccoid forms, is usually sufficient to put one on his guard. Some forms of the colon bacillus may show coccoid forms in culture also when grown on albuminous media. On transfer to ordinary agar the coccoid forms become less in number. Gram-negative forms of the staphylococcus (probably degenerated cocci) are found in secretions, and also in old cultures. The possibility of mistaking such Gram-negative cocci for the gonococcus in films from urethral discharges has been emphasized by Keyes and others.

Characteristics of the Gonococcus.—The gonococcus as seen in gonorrheal secretions from mucous membranes occurs in somewhat flattened pairs resembling coffee beans. Among the flattened forms may be found single cocci whose outline is more circular. In size the cocci vary, larger individuals measuring 1.6μ by .6μ. The apparent size of the organisms is influenced by the depth of the stain, lightly stained organisms appearing smaller than the more heavily stained. The size of the organisms does not of itself constitute an absolute diagnostic criterion (Koch). In cultures even greater variations in size are noted than in films of the gonorrheal pus, and the presence of many small round coccus forms, the outlines of
some of which are indistinct, together with the presence of irregularly rounded large forms (involution forms) present a picture rather characteristic of the gonococcus.

In gonorrheal secretions the intracellular position of the gonococcus is of definite diagnostic value, and with the exception of the meningococcus there is no other known organism which occurs to so great an extent within the leukocytes and other phagocytic cells. In film preparations from mucous membranes, as well as in preparations from serous effusions, there may be found many organisms lying free from the cells, usually in groups. This is more frequent very early in an infection, and during the later weeks or months when the acute purulent stage of the infection is past. Some writers have claimed that cases showing relatively small proportions of the organisms within the cells are more likely to develop severe general infection and metastases, but for this view there seems to be no confirmation (Koch).

The most satisfactory differential stain for the gonococcus is the Gram stain, the technique of which is familiar to all. Overheating of the films in the preliminary fixing should be guarded against. Care also should be taken that the stain is not allowed to evaporate, and that the decolorization with alcohol is thoroughly done. There has been much discussion as to the acquirement by older cultures of the gonococcus of relative Gram-positiveness. According to some workers of experience this may happen. Certainly, in some cultures at least, it does not occur, even after months and years of transfers. Unless one has had a wide experience with the organism, it is a safe rule to look with suspicion on any culture of the gonococcus which shows Gram-positive characteristics.

In cultures on ascites agar the gonococcus appears after 24 hours at 37°C as small grayish-white or grayish-blue, slightly raised, moist colonies about 1 mm. in diameter. Vigorous strains may show a somewhat more rapid growth, but unless the cultures are heavily seeded the colonies remain distinct for several days. The water of condensation contains small, round, whitish, or yellowish-white colonies .5 mm. in diameter and smaller; if these are numerous the fluid has a rather characteristic granular appearance. Cultures on blood agar present a similar appearance, except that the grayish tinge is somewhat more pronounced; hemolysis does not occur. Cultures of the gonococcus on blood agar are usually easily distinguished macroscopically from those of most other organisms such as the staphylococcus and hemolytic streptococcus. Non-hemolyzing streptococci and a small Gram-positive bacillus frequently met with in genital secretions appear in cultures on blood agar somewhat like the gonococcus colonies. The meningococcus may be found rarely in genital secretions, and requires further study of its growth on special media; acid is formed in media containing dextrose and usually in those containing maltose, while maltose is rarely fermented by the gonococcus; in gen-
eral the colonies grow more rapidly than do those of the gonococcus. So also micrococccus catarrhalis is to be distinguished, particularly by its more profuse growth and the readiness with which it thrives on plain agar. The colon bacillus grows profusely on albuminous media, and culturally its colonies are not easily confused with those of the gonococcus. In Gram-stained films there occur many diplococccoid forms, some of which taken alone might be mistaken for the gonococcus, but the presence of bacillary forms is distinctive. If there is still doubt as to whether the culture is a mixed one of gonococcus and colon bacillus, subcultures made simultaneously on plain agar and blood or ascites agar will usually decide the question. The cultures should be plated out.

In rare instances Gram-negative cocci occur in normal genital secretions and certain of these organisms have been called pseudogonocococcus. It is undoubtedly true that such Gram-negative cocci may be occasionally found, but when they are grown in cultures these are readily distinguished from true gonococci. Certain of these organisms have promptly become Gram-positive, which fact suggests the possibility that their Gram-negative qualities in film preparations of the secretion may have been an evidence of individual degeneration, such as is noted in certain older individuals in colonies of the staphylococci. Other instances of such Gram-negative cocci may be infections with micrococccus catarrhalis.

In cases where doubt arises as to the nature of the organisms in a secretion properly made cultures will decide the presence or absence of the gonococcus. For a further discussion of this question see Koch (33).

**Autolysis of the Gonococcus.—**For a number of years there was lively discussion as to whether the toxicity of cultures of the gonococcus was due to toxins secreted by the organisms, or whether it was derived from substances set free only by the death of the organisms. The weight of opinion favored the latter view, and this position has been further confirmed by the more recent studies of the changes which gonococci undergo when suspended in various fluids such as water, salt solution, or alcohol.

Suspended in salt solution gonococci are partially dissolved, so that the number of organisms in a unit quantity of suspension grows progressively less from hour to hour. The staining qualities of the organisms are also modified, and after prolonged exposure to salt solution many organisms are visible only as shadows. The filtrate of such a suspension contains protein in amounts increasing from hour to hour. The filtrate also has an inhibitive action on the growth of gonococci on media to which it is applied. (McClintock and Clark, 42.)

These changes in suspended gonococci leading to their dissolution are believed to be due to an active ferment present in or about the organisms. This tendency to rapid digestion or autolysis is exhibited by meningococcus and gonococcus.
Tricresol in 4 per cent. solution delays autolysis somewhat; tricresol and heating for one hour at 60° C. or above preserve the staining qualities of the organisms, as will also heat alone at 70° C. for one-half to one hour. Alcohol in strengths of 50 per cent. and 95 per cent. hinders autolysis; more dilute solutions have little effect. (McClintock and Clark, loc. cit.) Dilute acids delay autolysis. (Warden, 75.)

Colonies of the gonococcus show very early faintly staining organisms, with many cells of irregular outline. Strains recently isolated show a greater tendency to early autolysis, both in culture and in suspension, than do older stock strains.

**IMMUNITY IN GONOCOCCAL INFECTIONS**

Many years of clinical observation have failed to demonstrate any definite natural immunity in man to gonococcal infection. Certain differences in local susceptibility appear with advancing age, such as the relative freedom from infection of the adult vaginal mucous membrane, as compared with the frequency of infection of this structure in childhood. Nor is an acquired immunity of mucous membranes usually demonstrable, although the experiments of Jadassohn (see below) indicate that even mucous membranes may become refractory to infection. One attack of gonorrhea does not protect against a second infection, nor does a long-standing infection of mucous membranes prevent the occurrence of metastases.

The disease presents, however, certain peculiarities of location and course which may be interpreted as evidences of immunity. The infection is primarily one of the mucous membranes, the cells of which are in less intimate contact with the fluids of the body than are those of the deeper tissues, so that a relative insusceptibility or immunity to infection of the deeper tissues need not be shared to the same degree by the mucous surfaces, and conversely an infection of these surfaces may not stimulate the development of general immunity in the rest of the body. As pointed out by Koch and by Jadassohn (32), two clinical facts argue strongly for the view that gonococcal infections in man tend to produce immunity in the subject of the infection. When epididymitis, arthritis, or other evidence of general infection appears during the course of a urethral gonorrhea the local urethritis frequently ceases abruptly, and in fatal cases post-mortem examination may show little or no local evidence of the antecedent gonorrhea. This phenomenon has been explained on the ground that the high fever accompanying the general infection acts unfavorably on the organism in the urethra, and leads to its death. Such an hypothesis fails to take into account, however, that some cases presenting these phenomena show very little fever, and that in the distinctly febrile cases in
which the local discharge has disappeared viable gonococci may be occasionally obtained from the blood, and frequently from the joints.

The second clinical fact is that cases of urethral gonorrhea heal spontaneously after a period of a few days or weeks. This has been explained on various assumptions, such as alteration of the cells of the mucous membrane, or exhaustion of food supply of the gonococcus, or changes in the organisms themselves by which they become less resistant to unfavorable conditions of life, or become less virulent. The latter assumption of a decrease in virulence has no value as an argument against the existence of an increasing immunity in the host, for virulence is a relative state, and is dependent not only on the viability and activity of the invader, but also on the presence or absence of protective forces of the host. The subject of a recently healed and symptomless urethritis may harbor viable organisms which may be obtained by cultures, and may give rise to an acute infection in a second individual. Wertheim inoculated a man suffering from chronic gonorrhea seven times with cultures of the homologous organism without producing any change in the patient. The same organism produced an acute gonorrhea in a second individual, and when isolated from the second case and reinoculated into the first produced an acute gonorrhea. Wertheim interpreted this result as an indication that the passage of the organism through a second individual enabled it to act, when reintroduced into the first host, as a heterologous strain, which could now overcome the partial local immunity of the mucous membranes.

Jadassohn agrees that certain cases fall into the above class described by Wertheim, but maintains that many cases of chronic urethral infection exist which are immune to further infection either by homologous or heterologous strains.

In the light of recent studies on the serological changes to be described later the discordant opinions as to the presence or absence of immunity in the subjects of gonococcal infections of the mucous membranes may be in part explained on the ground of failure to recognize that immunity, if present, need not conform in point of duration to the immunity seen in certain other diseases. There is a growing mass of evidence that immunity does develop in gonococcal infections, but that it is of comparatively short duration, and may fluctuate greatly within short periods of time. The acquirement by the gonococcus of resistance to the defensive forces of the host (serum-fastness) may also be a factor favoring chronicity of the infection.

Infections of mucous membranes, such as that of the urethra, may remain confined to these structures so far as clinical signs indicate, but even in the apparently uncomplicated cases regional lymph nodes become enlarged and furnish evidence that the effects of the process are not confined to the external surface of the body. The frequency of involvement of the posterior urethra and prostate in gonorrhea needs no emphasis.
Studies of the fixation of complement indicate that immune substances in the blood as shown by this method appear usually only after the lapse of three or four weeks in urethral gonorrhea, although reactions have been obtained within a shorter time in some instances. This early appearance of the reaction of complement-fixation is probably associated with an unusually early involvement of deeper tissues.

The recurrence of gonococcal metastases such as arthritis, in the same or new joints, argues for an immunity of temporary character.

**Allergic Reactions.**—Following the inoculation of gonococcal vaccines or gonococcal protein in persons suffering from gonococcal infections, certain phenomena appear which resemble those seen after the introduction of tuberculin in tuberculous individuals. In patients suffering from gonococcal arthritis the inoculation of vaccines is frequently followed by a local reaction consisting of swelling and hyperemia at the site of inoculation of the vaccine, a focal reaction in the joints, with increased pain and tenderness lasting 24 to 48 hours, and a general reaction with malaise and increase in fever and leukocytosis, appearing a few hours after the inoculation and persisting 24 to 48 hours. These reactions have been studied by Irons (30), Bruck (10), Reiter (51), and others, and are of value in gauging the interval and dose of vaccines in treatment. They have also a limited diagnostic value which will be considered in a later paragraph.

Cutaneous allergy has been studied by Bruck and by Reiter and others using cutaneous and intracutaneous inoculations of gonococcus vaccine; by Irons (31) using glycerin extracts of the gonococcus inoculated by the method of von Pirquet. In patients suffering from gonococcal infections such as arthritis the cutaneous inoculation of gonococcal protein is followed in a few hours by the development of a papule with a surrounding area of hyperemia. The reaction persists for 24 hours, by the second day begins to fade, and by the third day may disappear. More pronounced reactions persist longer, and may be visible after five or six days. In studying this reaction observations should be made at the end of 24 and 48 hours.

**Diagnostic Value of Allergic Reactions.**—While these manifestations of altered reactivity to gonococcal protein in patients suffering from gonococcal infections appear to be, in part at least, specific, there are several factors which interfere with their utility as reliable diagnostic aids.

The general and focal reactions following inoculations of gonococcal vaccines appear to have some diagnostic value, although the studies of a number of men have indicated that the applicability of the test is limited by several considerations. The vaccine used for the test may vary in activity, according as it is prepared from old or freshly isolated strains of gonococci, and the dose must be correspondingly increased or decreased. The earlier work was done with relatively old strains, and 100- to 500,000,-
000 cocci were used at a dose. When freshly isolated cultures are used, the dose recommended is usually much smaller—5- to 50,000,000. The activity of each lot of vaccine must be determined.

Another factor which may lead to non-specific reactions in normal individuals is the tendency of vaccines prepared from freshly isolated strains to undergo rapid autolysis, by which the vaccine becomes more toxic. When this toxic vaccine is introduced into non-gonorrheic persons it may give rise to a local hyperemia and other non-specific symptoms, which may simulate a reaction.

In the third place, not all subjects of metastatic gonococcal infection exhibit reactions, even when the infection is extensive, and, furthermore, the degree of allergy seems to vary from time to time in the same individual.

Cutaneous reactions are subject to the same sources of error as are subcutaneous tests. Furthermore, apparently healthy individuals are occasionally met with whose skins are susceptible to any irritant. Inoculations of various bacterial proteins such as those from the colon bacillus, the staphylococcus, or of proteins derived from animal sources, or even of glycerin, may produce hyperemic and papular reactions which are clearly non-specific. The desirability of an easily applicable and reliable test for gonococcal infection has seemed to warrant an extended study of gonococcal allergy. As already noted, the writer has observed a definite alteration of reactivity to gonococcal protein in gonococcal infections, but this condition of allergy is not sufficient to establish the reliability of a test as a diagnostic measure. One of the chief difficulties lies in devising a method for the preparation of a reliable antigen which shall be non-toxic for non-gonorrheic persons and at the same time give reactions in infected cases.

Thus far allergic tests in gonococcal infections have often afforded information of some diagnostic value, but their presence or absence should not be regarded as an absolute criterion of the presence or absence of gonococcal infection.

Bruck (11) has utilized the reactions which follow the inoculation of vaccine to determine the size and interval of subsequent doses. He noted a focal reaction in epididymitis. Van de Velde (74) obtained reactions of diagnostic value in pelvic infections by inoculations of 10- to 40,000,000. Kutner and Schwenk (36), using doses of 5- to 40,000,-000, obtained reactions of diagnostic value in arthritis. Angenstein (1) saw a number of cases in which he was unable to obtain a reaction, but believes that the reaction is of value when obtained. Farkas (15) saw local and general reactions with arthigon (gonococcal vaccine, Bruck), in doses of .5 to 2 c. c. In many cases the temperature increased 1° to 2°, the joint became more swollen, with increased pain. He regards the local reaction also as of differential diagnostic value. Fromme (20) is inclined
to regard the local and focal reaction in pelvic infections as of little value, but attributes some diagnostic importance to the rise in temperature which occurs in freshly infected cases. Heynemann (27) concluded from a study of 26 cases of pelvic infection, including 6 cases of gonococcal salpingitis inoculated with arthigcon, that any sweeping conclusions as to diagnosis cannot safely be drawn from the reactions.

Hauser (23) reviews the diagnostic use of vaccine in pelvic infections and from his own results concludes that (1) a positive focal reaction and (2) a positive local reaction with coincident positive general reaction speak for gonococcal infection; a negative reaction in no wise excludes gonococcal infection. Hauser's material consisted of 95 cases of pelvic disease. In 21 cases the gonococcus was demonstrated, and in 15 of these there was a definite adnexal involvement. In all 21 cases a positive reaction was obtained with vaccine. Of 9 control cases in which gonorrhea could be excluded 8 gave negative reactions, and 1 case showed a local reaction, without general or focal symptoms.

Schultz (57) noted in addition to general reactions frequent focal reactions in cases of epididymitis and arthritis with only occasional local reactions. These cases were treated with intramuscular injections.

Cutaneous reactions have been studied by Shattuck and Whittemore (61). They were unable to obtain reliable results, and conclude that while certain features of the reactions, notably the papule formation, are probably specific, the results do not justify the use of the reaction as a diagnostic measure. Sakaguchi and Watabiki (52) made cutaneous tests with the gonococcus toxins and with extracts prepared in a manner similar to Koch's old tuberculin. With the latter they obtained positive results in a small proportion of cases, most of which were in patients with metastatic lesions. They do not regard the reaction as of practical value.

Finkelstein and Gershon (17) in a study of cutaneous reactions obtained positive results in 33 per cent. of acute cases and 91.7 per cent. of chronic cases of gonorrhea. They also obtained a positive reaction in three cases in which gonococcal infection was not demonstrable.

These reports, fairly representative of the results obtained by others, suggest that the methods of preparation of gonococcal products may modify the degree of autolysis of gonococcal protein, and this factor may account in part at least for the variable results. The difficulties met with in the preparation of suitable antigens, together with the variability in reactivity of infected and normal individuals, seriously interfere with the practical diagnostic value of allergic reactions in gonococcal infections, and the results of these tests must be interpreted with due allowance for the several sources of error. For the present they have at most only a confirmatory value.

**Serologic Reactions.**—**Agglutinins and Precipitins.**—Bruckner and Christeanu, Vannod, Torrey, and others have found specific agglu-
tinins and precipitins in the blood of animals, such as the horse, rabbit, and sheep, immunized by repeated inoculations of the gonococcus. Torrey (66-69) made a very extensive study of the agglutinins in rabbits, and obtained immune sera, one of which agglutinated the homologous strain of gonococcus in a dilution of 1-700,000. He found that, on the basis of agglutination reactions, strains of gonococci may be divided into several groups. Antigonococcus sera, with the exception of one serum, agglutinated the meningococcus only in very low dilutions. Other workers have corroborated Torrey's findings, but are inclined to lay less stress on the differences between the several groups of gonococcus.

Wildbolz and Bärmann obtained agglutination of gonococci by human serum from a case of epididymitis. Other workers have been unable to demonstrate agglutinins in human infections. The agglutination reaction has no value as a means of diagnosis of gonococcal infection in man. Finkelson and Gershun (17) examined 24 acute and 36 chronic cases of gonococcal infection for agglutinins, with uniformly negative results.

Opsonins.—Immune opsonins for the gonococcus develop in subjects of gonococcal infection, and the opsonic index has been used as a means of diagnosis. Abnormally low or high indices have been interpreted as an indication of the presence of gonococcal infection. By repeated determinations an opsonic curve may be plotted and employed as a guide to treatment by vaccines. It is generally conceded, however, that in cases suited to treatment by vaccines opsonic determinations may be economically and efficiently replaced by observations of clinical symptoms.

Complement-Fixation.—Immune sera derived from animals and from human subjects of gonococcal infection contain substances (amboceptors) which, in the presence of antigen (derived from the gonococcus), fix complement. For the technique of the reaction see page 137. This reaction has assumed considerable importance in the diagnosis of gonococcal infections. The test, like the Wassermann test for syphilis, requires a considerable degree of skill for its performance, and adequate controls are essential. It is specific in that, so far as is known, the antigen must contain gonococcal protein. This specificity does not, however, remove all the difficulties of interpretation of the test. The substances giving rise to the reaction in an immune serum may vary from week to week, so that a negative test does not exclude gonococcal infection. In the early weeks of a urethral infection negative tests are usually obtained, although an early metastasis may hasten the appearance of the reaction in certain cases. The reaction has been suggested as a test for the cure of the deeper local complications of gonorrhea, such as prostatitis and posterior urethritis. The results of the test in these cases have agreed in the main with those obtained by painstaking examination of the secretions by staining and by cultural methods.

In arthritis and other metastatic lesions a positive reaction is ob-
tained in a considerable proportion of cases, and when such positive re-
actions are obtained they are of confirmatory diagnostic value.

Negative tests in known positive cases of gonococcal arthritis are not
infrequently met with. The reaction may vary from positive to negative
in a case of arthritis within a few days, so that one negative test does
not necessarily exclude the gonococcus as the etiological factor in a doubt-
ful case. A persistently negative reaction, however, argues against the
gonococcal origin of an arthritis. Negative tests have been obtained also
following the use of vaccines.

Schwartz and McNeil (59) summarize an extensive study of com-
plement-fixation reactions as follows:

(1) A positive reaction denotes the presence or recent activity in the
body of a focus of living gonococci.

(2) A negative reaction does not exclude gonococcus infection, but,
for the reasons stated, should be accorded considerable importance.

(3) A strong positive reaction is not to be expected earlier than
about the fourth week, and then only in very acute cases with some com-
plication.

(4) A positive reaction is not obtained if the disease is limited to
the anterior urethra.

(5) A positive reaction does not entirely disappear until seven or
eight weeks after cure.

(6) If a strong positive reaction is obtained seven or eight weeks
after apparent clinical cure the patient should be looked upon as still
harboring gonococci.

(7) In chronic cases isolation of the gonococcus in culture is the
only absolute bacteriological proof of gonococcus infection.

(8) The technique of a complement-fixation test is simpler than
that of isolation of the gonococcus in culture, and the possibilities of
error are less.

(9) In cases regarded clinically as postgonorrheal a positive reaction
is obtained in 31.4 per cent.

(10) In 62 cases of chronic prostatitis giving a history of gonococ-
cus infection within three years, a positive reaction was obtained in 54.8
per cent.

(11) In 165 cases looked upon as clinically cured for at least three
months a positive reaction was obtained in 13.2 per cent.

(12) In women a positive reaction is probably not obtained unless
there is at least some involvement of the cervix.

(13) On account of the unreliability of the bacteriological diagnosis
of gonococcus infection in women, the complement-fixation test should
prove of special usefulness in gynæcoligical conditions.

The following table by Schwartz (58) shows the relative frequency
of occurrence of the reaction in his series of arthritis:
A positive reaction is obtained in a certain number of cases of gonorrhea where bacteriological examination fails. This is especially the case in women.

A negative reaction does not exclude gonococcal infection, but is to be given some weight on account of the reasons detailed earlier.

It is to be remembered that gonorrhea is a common infection in both sexes. This fact should not be lost sight of in interpreting a positive result in connection with any given case of arthritis. A person may suffer from two infections; for example, acute rheumatic fever and gonorrhea, as shown in the preceding records.

Interpreted, however, in the light of the clinical history and clinical findings, it seems to us that the complement-fixation test should prove an addition to our means of diagnosis between gonococcal arthritis and other forms of arthritis of obscure etiology.

Lenartowicz (39), using a polyvalent antigen, obtained a positive reaction oftenest in cases in which gonococcal infection was extensive. In local gonorrhea he obtained negative results. He concludes that the test is of value only in cases with complications, that it is specific, and is negative in healthy persons.

He obtained positive complement-fixation reactions in the following groups of cases:

<table>
<thead>
<tr>
<th>Condition</th>
<th>Per Cent.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adnexitis</td>
<td>83</td>
</tr>
<tr>
<td>Arthritis</td>
<td>80</td>
</tr>
<tr>
<td>Epididymitis</td>
<td>52</td>
</tr>
<tr>
<td>Bartholinitis</td>
<td>52</td>
</tr>
</tbody>
</table>

McNeil (44), from a study of between 3,000 and 4,000 sera from persons suffering from all stages and varieties of gonococcal infection,
concludes that the results of the test seem to justify the assumption that a positive reaction denotes the presence of recent activity in the body of a focus of living gonococci. In eight cases of vulvovaginitis in children, six of which had no discharge at the time of examination, a positive reaction was found in all. McNeil suggests that infantile vaginitis may be more than a local infection and that the infection may be active after all local manifestations have disappeared. Smith (62) observed weakly positive reactions in cases of vaginitis which one year previously had given negative tests following apparent cure, with no subsequent discharge. He suggests the possibility that the later tests may have been more delicate, owing to improvements in technique (see below).

In studying the reactions in a series of cases of scarlet fever in which routine Wassermann tests were being made, H. K. Nicoll and the writer occasionally noted a delay in hemolysis in the gonococcal test tubes similar to that observed in the series of tubes set up for the Wassermann test.

In general our experience leads us to believe with others who have used the test that when properly performed with adequate controls a positive complement-fixation reaction affords valuable confirmatory evidence of the presence of gonococcal infection. A negative reaction repeatedly obtained at intervals of four to seven days argues for the absence of active gonococcal infection.

As a test for the cure of gonococcal infection a negative reaction is of some value, but should be repeated before great reliance is placed on the result. In metastatic gonococcal infections the complement-fixation reaction may shift from positive to negative within short periods of time. This variation in complement-fixing power of the blood supports the view that gonococcal infection produces an active immunity, but that this immunity may be of very brief duration.

In the interpretation of "weak" reactions some care is necessary, and where such reports are received the test should be repeated. The possibility of "group" reactions whereby infections by allied organisms may give rise to faint reactions with gonococcal antigen must also be considered. This question requires further study. In sera giving strong complement-fixation reactions with polyvalent gonococcal antigen confusion is not likely to arise in the interpretation of the tests.

**THE TREATMENT OF GONOCOCCAL INFECTIONS BY SERA AND VACCINES**

In gonococcal infection, the course of which unmodified by treatment is so varied, both as to time required for cure and as to the extent and duration of complications, the formation of a judgment as to the value of any method of treatment is a matter of considerable difficulty. Large
series of cases with adequate controls must be studied, and even then a clean-cut statement may be impossible.

At the outset it should be understood that specific methods of therapy are not to be used to the exclusion of other rational measures but rather as an adjunct to them. Most of the complications of gonococcal infection bear the same relation to the primary disease or its local extensions that the chronic joint lesions of other infections bear to the primary focus in tonsils or other tissues of the body. With this relation in mind efforts to influence the course of gonococcal lesions by specific measures should be combined with other rational treatment calculated to eradicate the source of the recurrent metastatic lesions. Prostatic massage, the surgical treatment of pelvic disease, and measures for the cure of the lesion in the genital tract are important. Attention must also be paid to general hygienic conditions, an adequate diet, the enforcement of rest in cases of acutely inflamed joints, and the application of suitable surgical treatment in cases in which this is demanded.

Further, it must be recognized that, granting the efficacy of vaccines in the establishment of immunity, they cannot be expected to promote absorption of bony exostoses, or the solution of calcified or fibrous adhesions, nor to stop the progress of anatomical changes in joints which, irritated by single or repeated infections, advance by reason of mechanical irritation resulting from movement and the tension of muscles and tendons. In such cases the most that could be hoped for from specific therapy would be the prevention of reinfection; after the removal of the infectious element of the arthritis whatever healing takes place must depend on other factors such as relative rest and the tendency of such lesions to heal to a greater or less extent, when the mechanical causes of the conditions are remedied. In arthritis of infectious origin the power of repair manifested after the removal of the infectious cause is often remarkable.

Before turning to the clinical evidence for and against the efficacy of specific therapy in gonococcal infections, we may summarize the evidence available from studies of the reactions of immunity and from animal experimentation.

The inoculation of suspensions of the gonococcus into animals gives rise to specific agglutinins, precipitins, opsonins, bacteriolysins, and complement-fixing substances in the serum of the inoculated animals. In man the reaction of complement-fixation is demonstrable in a considerable proportion of cases of gonococcal infection. The degree of the reaction fluctuates spontaneously, and also is modified by inoculations of gonococci (M'Donagh and Klein, 43; Finkelstein and Gershun, 18). In man the inoculation of gonococcal protein produces reactions, local, focal, and general, in the subjects of general gonococcal infection, exceeding in frequency and degree the reactions produced by similar doses in non-infected
persons. Animals may be immunized against the gonococcus, but the natural insusceptibility of laboratory animals to gonococcal infection has thus far prevented the experimental demonstration of the effects of specific therapy on established infections.

**Gonococcal Vaccines.—Preparation.**—The methods of preparation of vaccines from cultures of the gonococcus are essentially the same as those employed for other organisms. Cultures may be grown on blood agar, ascites agar, Thalman’s agar, or salt-free veal agar for 24 or 48 hours at 37° C. The shorter period of growth of 24 hours yields a suspension of cocci in which there is less clumping than in older cultures, and accurate standardization is more easily obtained. The cultures are washed off with distilled water or salt solution, to which .5 per cent. phenol has been added. The suspensions should be standardized immediately, as autolysis of the gonococci proceeds rapidly, and a delay of an hour may result in figures much lower than those obtained immediately after making the suspension.

Standardization may be performed by Wright’s method by the use of the ordinary pipettes and counting chamber employed in the examination of blood. The amount of gonococcal protein may be estimated by weight after evaporating the water from suspensions in distilled water, but this method is more applicable to the preparation of large quantities of the product. Methods involving the comparison of turbidity or density of suspensions with permanent control suspensions are less suited to the standardization of gonococcal than to suspensions of certain other organisms.

Sterilization by phenol or other related antiseptics is effective and seems to be preferable to heat. After standardization the vaccine is diluted with salt solution containing .5 per cent. phenol. Sterility is determined by a cultivation test. The vaccine is preserved in sealed tubes in the cold.

Polyvalent vaccines derived from a number of cultures of the gonococcus are usually employed unless an autogenous culture is available.

**Dosage.**—The dosage recommended in gonococcal infections has varied greatly, from 1- to 1,000,000,000 being advised by different workers. This wide variation is due largely to the fact that cultures of varying ages have been used. If old laboratory strains are employed the larger doses of 100- to 500,000,000 may be employed. When young, freshly isolated cultures are used smaller doses of 5,000,000 or more are advisable. The optimum dose for a given preparation may be determined by graduating the amount from small to larger quantities. The sensitiveness of the individual patient also is a large factor in determining the optimum dose. Fresh preparations are preferable to those which have been in stock many months.

Nicolle and Blaizot (46) have described a method of preparing vac-
TREATMENT BY SERA AND VACCINES

eine which is said to be atoxic and stable. They employed a culture
medium containing urea .40, glucose 2.0, ammonium phosphate .05,
sodium chlorid 1.0, agar 1.5, beef broth 100. One-half c. c. of rabbit
serum was added to each tube containing 5 c. c. Twenty-four-hour cul-
tures of the gonococcus on this medium were washed off with a 7 per cent.
aqueous solution of sodium fluorid and repeatedly centrifuged. To one
part of this emulsion were added 9 parts of a similarly prepared emulsion
grown on the same medium, without serum, of an organism which the
authors term "synocoque." This latter organism is a Gram-positive coccus
resembling morphologically the gonococcus. The mixture of emulsions is
standardized so that 500,000,000 are contained in 1 c. c. The initial dose
is 1 c. c. diluted in salt solution before inoculation.

Nicolle and Blaizot do not state the origin of this second organism
contained in their vaccine, but from the brief description given it is sim-
ilar to the staphylococcus frequently met with in urethral discharge in
gonorhea. Warden (76) has suggested that this organism, referred to
by him as "staphylococcus urethrae," may play an important etiological
part in the discharge in gonorrhoea.

Sensitized Vaccines.—Sensitized gonococcal vaccines are prepared
after the method of Besredka by treating thick suspensions of gonococci
with gonococcal serum. The serum is added to the suspension which has
been standardized, and after a period of exposure to the serum the or-
ganisms are removed from the mixture by centrifugation, resuspended in
salt solution, and again centrifuged. The supernatant fluid is discarded,
and the sensitized gonococci freed from the serum taken up with a known
volume of fresh salt solution. The period of exposure to serum has varied
in the hands of different workers. M'Donagh and Klein (43) allowed
the mixture to stand 12 hours at room temperature. In view of the rapid
autolysis of the gonococcus when suspended in salt solution, it would seem
that a shorter exposure at a lower temperature would be advisable.

The advantage claimed for sensitized gonococcal vaccines is that larger
doses may be given without producing severe reactions. M'Donagh and
Klein used sensitized vaccines in doses of 20-, 50-, and 100,000,000 on
three successive days. Later they used 200-, 300-, and 500,000,000 on
successive days.

Tests of the serum used for sensitizing have shown that, unless enor-
mous quantities of gonococci are used with relatively small amounts of
serum, the serum loses but little complement-fixing power. Small amounts
of immune serum suffice to sensitize considerable quantities of gonococci.
Thus, when 1 c. c. of serum was used in sensitizing 1 c. c. of gonococcal
emulsion containing 1,000,000,000 cocci, there was no apparent loss of
antibody as determined by a comparison of the complement-fixation of
the serum before and after exposure to the emulsion. When 8,000,000,000
cocci were added to 1 c. c. of serum it showed a subsequent diminution
of complement-fixation (M'Donagh). It is of course possible that substances other than those capable of giving rise to the fixation of complement may be concerned in the process of sensitization.

Various immune sera have been used for sensitization, among which may be mentioned those derived from the rabbit, horse, and man. Human serum is theoretically preferable, and such sera with fairly high antibody titre, as determined by complement-fixation, may usually be found.

Commercial Preparations of Gonococcal Vaccines.—The commercial gonococcal vaccines in this country are supplied in various strengths, according to the number of organisms present in a unit volume at the time of manufacture. This number is stated in millions on the label of the container.

Bruck gave the name "arthigon" to a vaccine which he prepared and which appears frequently in the reports of treatment from German clinics. The average dose is .5 c. c. to 2.0 c. c., but the number of organisms in these quantities is not usually given. Schindler states that .5 c. c. of arthigon contains 40,000,000 gonococci.

Reiter's vaccine contains 5,000,000 per c. c. A new vaccine by Reiter (A. 10), as described by Hauser (23), is prepared from young cultures washed from the surface of the medium with distilled water incubated 2 days at 37° C. and diluted with salt solution to which phenol has been added. The suspensions are not heated. One c. c. of the vaccine contains 70- to 100,000,000 gonococci.

The use of extracts prepared by the digestion of suspensions of the gonococcus by pancreatin have been suggested (Hirschfelder, 28).

Regulation of the Size and Intervals of Inoculations.—Determinations of the opsonic index have been discarded as a routine procedure, and the clinical reaction which follows the inoculation has been substituted as the guide to treatment. Local reactions at the site of inoculation have some value as indicating the activity of the vaccine. Bruck believes that in the treatment of arthritis and epididymitis a slight temperature reaction of 1° to 2° F. is indicative of efficiency of the dose. Certainly more intense reactions are inadvisable. The degree of reaction allowable must be determined by a study of the individual case, but in general it is safe to restrict the dosage so that the clinical changes in the patient immediately following the inoculation are relatively slight. The intervals of inoculation vary from 2 to 7 days or longer.

The reaction of complement-fixation has been employed as an index of the efficiency of inoculations. M'Donagh and Klein observed changes in the degree of the reaction following the inoculations of vaccines, and attributed the changes to the inoculations. Their tables support this view, although it must be remembered that in some instances the reaction of complement-fixation may fluctuate from positive to negative, or vice
versa, within short periods of time, independently of specific therapy. For this reason caution is necessary in the interpretation of these reactions.

A comparison of the serum reactions seen after the inoculation of sensitized gonococcal vaccines with those observed after sensitized typhoid vaccines is of some interest. In experiments on typhoid vaccination the sensitization of vaccines has resulted in a decrease in the amount of agglutinins formed. Broughton-Alcock (8) found no fixation of complement in patients treated with sensitized vaccines, while in four patients inoculated with Leishman's vaccine (unsensitized) he found a positive fixation of complement on the eighth day after inoculation.

Examination of the tables of M'Donagh and Klein shows that changes in fixation of complement appear after sensitized as well as after unsensitized vaccines in patients suffering from gonococcal infection.

It cannot be assumed of course that the treatment of gonococcal vaccines with immune serum will necessarily produce the same changes in their antigenic qualities as are produced by corresponding treatment of typhoid vaccines. Moreover, Broughton-Alcock's experiments were performed on healthy persons, while the above experiments with the gonoccus were made on persons already infected. This question requires further study.

TREATMENT BY VACCINES

This phase of the treatment of gonococcal infections may be conveniently considered, first, from the point of the original infection of mucous membranes, and, second, the treatment of the complications which include local extensions (prostatitis, tubal infections, epididymitis, and the metastatic lesions, such as arthritis, periostitis, tenosynovitis, and iritis). Inoculations of vaccines are usually given subcutaneously, but intramuscular inoculations may be used. Intravenous inoculations have been recommended, but for the average case the subcutaneous route seems preferable.

Infections of Mucous Membranes

Urethritis.—There have been a few reports of beneficial results in the treatment of urethral gonorrhea by gonococcal vaccines, but the majority of workers who have had wide experience agree that vaccines produce little if any demonstrable effect in shortening the course of the disease. Bruck (10), Schindler (53), Föckler (19), Pollock and Harrison (49). In old cases a temporary increase in discharge has been noted after vaccines, and this procedure has been suggested as a method of determining the cure of such cases.

Active immunization in gonorrhea has been suggested as a means of preventing complications by increasing immunity, and thus anticipating the possible spread of the infection from the primary focus. Pollock
GONOCOCCAL INFECTIONS

and Harrison report the results of this treatment in a limited number of cases in the English army.

Alternate patients suffering from gonorrhea without complications were given on admission to the infirmary an inoculation of gonococcal vaccine (25,000,000), and all patients, both inoculated and controls, were then passed into the hands of other medical officers, and received the same routine treatment. In all 84 patients were admitted during the investigation; to 42 of these a dose of vaccine (25,000,-000) was given on admission, and to 42 no vaccine at any time, unless complications occurred. Of the patients treated with vaccine 4 subsequently developed epididymitis, including 1 who developed this complication 18 hours after the injection. Of those who were not treated with vaccine 10 developed epididymitis, and 1 of these suffered also from very severe arthritis. Lieut. C. H. Harold's very careful notes on all the cases showed that in those treated with vaccine periurethral thickenings were not so marked, and the general course of the urethritis was milder, though the duration of the purulent stage was not markedly affected. In many of the cases an increase of the discharge, lasting a day, appeared to follow the vaccine, but there was no marked exacerbation of symptoms which would contraindicate its use in such cases.

Cruveilhier (12), using sensitized vaccines, thought the course of the disease was somewhat shortened, and noted no complications in treated cases.

Angenent (1) observed complications in 3 of 42 cases of acute gonorrhea treated by vaccines. Others have seen complications developing during treatment of acute gonorrhea by vaccines, but in the reports available in the literature this is apparently somewhat less frequent than in uninoculated cases. The choice of other procedures of treatment may also be a large factor in the frequency of development of metastatic lesions.

Other organisms, such as the staphylococcus, colon bacillus, and certain diphtheroid organisms, undoubtedly play a large part in the continuation of the urethral discharge, as also do the mechanical difficulties, such as strictures, which develop as a result of prolonged inflammation.

Vulvovaginitis.—Genital infections in little girls have been extensively treated by vaccines. Some observers believe that the course of the disease has been shortened, others that the method has been of no demonstrable value.

The determination of the value of vaccines by a comparison of the length of time required for the cure of inoculated and uninoculated cases presents almost insurmountable difficulties, for the length of time required for the cure of uninoculated cases may vary enormously, and relapses after apparent cure are frequent. Thus Birger (quoted by Pollock) found that the average time required for treatment of children with gonococcal infection was 80.4 days, although the actual time in different cases varied between 28 and 296 days.

It would appear that treatment by vaccines has not been clearly shown to shorten the period of treatment.
Schlingenberg (54) could not see any clear benefit from vaccines in genital infections in the adult female, and Föckler (19) arrived at similar conclusions in the treatment of cervicitis and proctitis.

Gonococcal Ophthalmia.—A limited number of cases of ophthalmia treated by vaccines have been reported, but, on the whole, the results do not appear better than in infections of other mucous membranes.

Summary.—The collected reports on the treatment of gonococcal infections of mucous membranes by vaccines present no clear evidence of their efficacy. As a prophylactic against metastatic complications they may have some value. Theoretically, bodily immunity should be shared to a slight degree by the mucous membranes and the tissues contiguous to them, but this immunity seems not to be regularly demonstrable by the clinical course of treated cases.

Treatment of Complications

The principal complications of gonococcal infections are arthritis, epididymitis, and pelvic infections in women. As indicated in the preceding section, the number of lesions possible in the course of gonococcal infection is large, and will include also endocarditis of the ulcerative type, as well as cases of sepsis, but these latter are met with much less frequently than the first three named. The relation of metastatic lesions, such as arthritis and iritis, to a temporary gonococcemia has already been pointed out.

Gonococcal Arthritis

Under this heading may be included tenosynovitis and the periosteal and periarticular localizations, which are often associated with arthritis. Bruck (11) has summarized the results reported by a large number of workers in America, Germany, and England up to 1912 as follows: "The favorable influence of gonococcal vaccines in the active treatment of the complications of gonorrhea, arthritis, epididymitis, and infections of adnexae has been emphasized by those who have concerned themselves with the subject." In evaluating reports in medical literature on the use of gonococcal vaccines in arthritis the same difficulties are met as in estimating the value of any other therapeutic method from clinical reports. Over-enthusiasm must be discounted and conclusions obviously unwarranted discarded. The articles of the past two years on this subject deal in the main with large series of cases, and are fairly conservative. The general impression seems to be that the course of gonococcal arthritis may in certain cases at least be favorably influenced by inoculations of gonococcal vaccines.

Kutner and Schwenck (36) conclude that cases of arthritis are benefited by vaccines. They used doses of 5- to 40,000,000. Murrell (45), using autogenous vaccines, saw good results in arthritis. Frost (21) ob-
GONOCOCCAL INFECTIONS

ained favorable results in arthritis and epididymitis with polyvalent vaccines. Föckler found vaccines of clearly demonstrable value only in arthritis. In epididymitis his results were not much better than by other methods of treatment. Farkas (15) had good results in arthritis with arthigon .5 to 2.0 c. c. (40- to 160,000,000: Schindler). With these doses he obtained local, focal, and general reactions. In many cases the temperature rose 1° to 2°, the joint became more swollen and tender, the overlying skin redder. In the cases treated there was no instance of ankylosis, and the duration of treatment was 10 to 24 days. He believes the local reaction has a differential diagnostic value. In chronic cases with no local reaction the treatment offers no prospect of cure. Papée (49) obtained reactions both local and general after inoculation of arthigon, and concludes that the treatment benefited arthritis; he noted no recurrences in the treated cases. Schultz (57) saw prompt improvement in 11 of 16 cases of arthritis, and concludes: (1) the treatment is specific, as shown by focal, local, and general reactions; (2) cases reacting with fever do better than those which show no reaction.

Baetz (4) treated 28 cases of gonococcal arthritis in laborers in the Canal Zone, with inoculations of 50- to 400,000,000 at intervals of two days. He concludes that vaccine treatment gave very good, sometimes brilliant, results in the majority of cases. Unfavorable results due to treatment were not noticed. The average number of days' treatment per man was 24. Block (5) saw improvement in gonococcal arthritis following the injection of typhoid vaccine as well as following gonococcal vaccine, and suggests that the improvement may be due to nonspecific effects of the reaction on gonococcus infection.

The limitations of the method have already been referred to. The most favorable cases are those in which lesions are subacute and in which secondary anatomical changes are limited. It should be obvious that in cases of long standing with disorganization of joint structure all that can be hoped for from specific measures is the prevention of new infections and that the repair of the joint, if at all possible, must come from other agencies. Joints and tendons whose function is limited by adhesions can receive no benefit from inoculations further than the prevention of new inflammation. It should be noted, however, that frequently disability is accentuated by periarticular edema, which subsides when the acute process giving rise to it heals. Finally there is a small group of chronic arthritides usually multiple, associated with more or less disorganization of joint structure resembling that seen in arthritis deformans, in which there is an associated chronic urethral and prostatic gonococcal infection, and in which the complement-fixation test in blood and joint fluids may be persistently positive, the cases of which fail entirely to respond to vaccines, either alone or combined with other methods of therapy. These cases may show progressive emaciation without fever, and sometimes develop a sub-
TREATMENT BY SERA AND VACCINES

acute or chronic nephritis with albuminuria and casts. Autogenous as well as polyvalent vaccines fail to elicit any lasting improvement. These cases usually show no reaction after inoculation.

M'Donagh and Klein, whose work has already been referred to, present some interesting studies of the complement-fixation test in relation to treatment by vaccines. They found that, while the inoculation of vaccines in normal or non-gonorrhoeal persons produced no change in the serum reaction, such inoculations into the subjects of arthritis or other gonorrhoeal complications produce marked changes, either converting a negative into a positive reaction, or vice versa. Their results furnish a strong argument for the specific effect of vaccines in altering the reactions of immunity. The following cases taken from the paper of M'Donagh and Klein illustrate:

Case I. Male, age 35. Severe case. Subacute posterior urethritis, chronic prostatitis and arthritis of both knees and ankles, with rheumatic pains about the right shoulder. Subcutaneous vaccines (freshly prepared, not autogenous).

<table>
<thead>
<tr>
<th>Injection of Vaccine Subcutaneously</th>
<th>Serum Test</th>
<th>Therapeutic Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before Vaccine</td>
<td>Forty-eight Hours After</td>
</tr>
<tr>
<td>First injection, 5,000,000</td>
<td>+++</td>
<td>−</td>
</tr>
<tr>
<td>Second, 10,000,000 (eighth day after first)</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Third, 15,000,000 (eighth day after second)</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Fourth, 25,000,000 (two weeks after second)</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Fifth, 50,000,000 (eighth day after fourth)</td>
<td>+</td>
<td>+++</td>
</tr>
</tbody>
</table>

Case II. Male. Case illustrates diagnostic value of gonococcal fixation test. Gonorrhoea twelve years ago. Syphilis two and a half years ago, treated with seventy-two mercurial injections. No signs or symptoms for about two years. Six weeks ago iritis of left eye occurred. Wassermann reaction negative. Gonococcal fixation test positive. Iritis cured with gonococcal vaccines without any antisypililitic remedies.

<table>
<thead>
<tr>
<th>Subcutaneous Vaccine</th>
<th>Serum Test Before Vaccine</th>
<th>Serum Test Forty-eight Hours After</th>
</tr>
</thead>
<tbody>
<tr>
<td>First injection</td>
<td>+++</td>
<td>−</td>
</tr>
<tr>
<td>Second, eighth day after first</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Third, eighth day after second</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Fourth, eighth day after third</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Fifth, eighth day after fourth</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Sixth, eighth day after fifth</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>
Gonococcal Iritis

Gonococcal iritis or uveitis may occur with arthritis simultaneously, or months or years afterward, or in rarer instances there may be no history of antecedent arthritis. In doubtful cases a careful history, a searching routine examination of the genito-urinary system, the specific immune reactions, including the reaction of complement-fixation, together with the absence of other forms of focal infection will furnish the evidence necessary to a diagnosis. It must always be remembered that iritis from other sources of infection may occur as well in a patient who is suffering from gonorrhea as in other patients. Some observers have seen focal reactions in the eye after inoculations of vaccines; others have not been able to note such reactions. Certain cases of iritis of the recurrent type have apparently received benefit from active immunization. Local treatment of the primary disease in urethra and prostate must not be neglected.

Epididymitis

Many series of cases of epididymitis are on record in which vaccines are credited with hastening the cure—Angenant (1), Kutner and Schwenck (36), Frost (21), Cruveilhier (12), Panoff (48), Schultz (56). This evidence is not so convincing as that offered by studies of arthritis, in that epididymitis frequently subsides with rest, external applications, and support to the parts affected.

Klause (33) has reported the results of treatment of 700 cases by polyvalent gonococcal vaccines, using subcutaneous and later intraglutheal inoculations. The dose varied from 10- to 100,000,000. Of 269 cases of recent epididymitis prompt healing was obtained in 245, or 91 per cent.; in 24, 9 per cent., the results were less striking. Of 94 old cases of epididymitis 81 per cent. were improved. Two or three inoculations were usually sufficient. Arthritic cases were also benefited by vaccines. Urethritis and prostatitis treated by vaccines showed no improvement greater than could be obtained by other methods. Complications were not less frequent than in uninoculated cases. Febrile reactions and focal reactions in closed cases were frequently noted after vaccines, but Klause believes that they are not reliable for diagnosis.

Pelvic Infections

In pelvic infections in women the action of vaccines has been studied from the diagnostic and therapeutic standpoint. Hauser (23) has reviewed this subject, and summarizes his own results and those of others in the treatment of pelvic infections. He believes that in recent cases and in those which still give a focal reaction to inoculations vaccines have a specific curative value; that inoculations must be carefully conducted to
avoids doing harm; that a positive focal reaction or a local reaction with positive general reaction speaks for the gonorrheal nature of the lesion, while a negative reaction by no means excludes gonococcus as the cause of the lesion. Favorable results were attributed to the action of vaccines by Hauser in 82.3 per cent., Illeinsius (24) in 80 per cent., Fromme (20) in 64.4 per cent., and Dembskaja (13) in 50 per cent.; complete recoveries, Sternberg and Jelkin (63) 87.1 per cent.; general average 70 per cent. Heymann and Moos (26) are inclined to the view that vaccines may have some value in recent pelvic infections, but warn against their indiscriminate use. In urethral and uterine infections and in old pyosalpinx vaccines were of no benefit.

Schottmüller and Barfurth (56) have recently made a study of the organisms found in inflammatory adnexal processes, and their figures have a bearing on the question of the specific treatment of such lesions. They made anaerobic cultures of secretions from pelvic lesions, and compared their results with those of the collected statistics by Hermann.

<table>
<thead>
<tr>
<th></th>
<th>Hermann—1900 1,000 Collected Cases</th>
<th>Schottmüller—1913 70 Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gonococcus</td>
<td>216 = 21.4 per cent.</td>
<td>5 = 6.3 per cent.</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>118 = 11.7 per cent.</td>
<td>14 = 17.8 per cent.</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>93 = 9.2 per cent.</td>
<td>20 = 25.3 per cent.</td>
</tr>
<tr>
<td>Sterile</td>
<td>578 = 57.7 per cent.</td>
<td>12 = 15.2 per cent.</td>
</tr>
<tr>
<td>Anaerobic organisms</td>
<td></td>
<td>28 = 35.4 per cent.</td>
</tr>
</tbody>
</table>

Schottmüller and Barfurth conclude that, while a large proportion of tubal infections are due to the gonococcus, there is a considerable number that are referable to anaerobic organisms. The presence of a gonococcal vaginal infection does not necessarily mean that the tubal process is gonococcal, and not anaerobic.

Summary.—The uselessness of treating non-gonococcal lesions with gonococcal vaccines is evident. The value of vaccines in gonococcal pelvic lesions is not clearly determined. If they are employed care should be taken that the dosage is not excessive, and that the hope of cure by specific treatment is not allowed to distract attention from other methods of local or surgical treatment that may offer greater prospects of prompt alleviation of the trouble.

Other Complications

A number of reports on the use of active immunization in prostatitis are available, in which the authors believe that improvement was more rapid than in control cases not inoculated. (Kutner and Schwenck, 38;
Panoff, 48.) Schindler (53) cautions against over-enthusiasm in attributing improvement to any one element out of several concerned in the treatment of a given case.

Cases of gonococcal sepsis have been reported, in which part of the improvement was attributed to inoculations of vaccines. There seems to be little theoretical evidence in support of such a conclusion. The writer has seen several such cases, in which gonococci were isolated from the blood, recover without specific treatment; of four fatal cases of gonococcemia two received vaccine and two did not. As a matter of fact, considering the frequency of gonococcal invasion of the blood, a fatal outcome is relatively rare, so that recovery from this condition is not a strong argument for any method of treatment.

**AntigonoCoccic Serum**

The sera of animals, such as the rabbit, ram, and horse, immunized by repeated inoculations of the gonococcus, contain agglutinins, precipitins, opsonins, bacteriolysins, and complement-fixing substances specific for the gonococcus, and have been used for the treatment of gonococcal infections in man. In most of the cases studied the serum has been used subcutaneously. In infections of the mucous membranes it is generally conceded that the serum has produced no beneficial effects. In arthritis and other metastatic lesions some observers have claimed good results, but their findings have not been generally confirmed. The consensus of opinion seems to be that the value of antigonoCoccic serum as it is usually given is not proved. In severe sepsis its use might be considered.

The relation of meningococcic infections to those by the gonococcus are of some interest in connection with this failure of antigonoCoccic serum to produce results. In meningococcic infections we have an infectious process with frequent bacteriemia, and a usual localization in the meninges with rarer arthritis. Antimeningococcic serum has very little if any demonstrable effect on meningal symptoms in fully developed meningitis when given subcutaneously or even intravenously. Subdural injections have a definite effect on meningeal infections, and intra-articular injections of serum a prompt curative action in meningococcic arthritis.

In gonococcal infections we have an infectious process with intermittent bacteriemia and frequent localization in joints. Given subcutaneously, serum has not produced satisfactory therapeutic results; when injected into infected joints improvement has been noted in some cases. The practical difficulty in the local treatment by serum lies in the fact that the arthritis is usually polyarticular and often periarticular, and the anatomical relations of the lesions do not admit of the successful introduction of serum into the foci of infection. The serious features of ordinary metastatic gonococcal lesions (excluding the cases of acute gonococ-
REFERENCES

For other references up to 1912 see Kolle u. Wassermann, Handbuch der pathogenen Microorganismen, 1913, iv, 709, 733.


5. Block. Cor.-Bl. f. schweitz. Aerzte., 1914, xliv, No. 44.


10. ——. Über specifische Behandlung gonorrhöischer Prozesse, Deutsch. med. Woch., 1909, nr. 11.

11. ——. Kolle und Wassermann, 1913, iv, 732.


34. Koch, J. Handb. der path. Mikroorg., Kolle und Wassermann, 1913, iv, 703.
38. Lassérre, P. Antimeningococcus Serotherapy of Gonorrheal Arthritis, Jour. de méd. de Bordeaux, 1913, xliii, 289.
REFERENCES

41. Lumière and Chevrotier. La Semaine méd., 1914, xxxiv, 44.
44. McNeil, A. Complement Fixation Test for Gonorrhea, Arch. of Ped., 1913, xxx, 657.
45. Murrell. Gonorrheal Rheumatism, Practitioner, 1912, lxxviii, 1, 34.
46. Nicolle and Blaizot. La Semaine méd., 1913, xxxiii, 497, 595.
49. Papée. Über die Vaccinbehandlung der gonorrhoeischen Komplika-
54. Schlingenberg. Vaccinetherapie by Vrouwelyke gonorrhoe Neder-
55. Schmutz. Sur les nouveaux traitements des epididymites aiguës 
blennorragniques of en particulier de leur traitement per le 
sérum antimeningococcique, Jour. d’urologie, Paris, 1913, iv, 
169.
56. Schottmüller and Barfurth. Zur Aetiologie der citrigren Adnexer-
Bd., 1 Heft, 45.
57. Schultz, J. H. Klinische Erfahrungen mit dem Gonokokken 
Vakzin Arthigon (Bruck), Deutsch. med. Woch., 1911, xxxvii, 
2331.
58. Schwartz. The Complement Fixation Test in the Differential Diag-
59. Schwartz and McNeil. Further Experiences with the Complement 
Fixation Test in the Diagnosis of Gonococcus Infections of the 
Sci., 1912, cxliv, 815.


67. ——. J. A. M. A., 1907, xlii, 918.


69. ——. Ibid., 1908, xix, 471.


71. Tyree. Internat'l Clinics, series 18, ii.


76. ——. Ibid., 1913, xiii, 124.


CHAPTER XXII

VACCINE THERAPY IN DERMATOLOGY WITH SPECIAL REFERENCE TO ACNE AND FURUNCULOSIS

J. FRANK WAUGH

Since Wright first applied the principles of vaccine therapy to furunculosis and sycosis it has been used in a number of other cutaneous disorders with varying results. It has its indications and is of unquestionable value in certain conditions when administered properly. However, it should not be used in every case where an infection is present. A folliculitis due to a staphylococcal infection, impetigo contagiosa, staphylococccia, etc., will clear up readily, as a rule, in a few days under suitable local treatment; and in no instance should local and general treatment be neglected or subverted to vaccine therapy.

Its value and beneficial results are most noticeable in chronic or recurrent types of staphylococcal infections such as pustular acne, furunculosis, sycosis vulgaris, and chronic or subacute dermatitis staphylococcia. In certain types of acne vulgaris the acne-bacillus vaccine has also proved its value; yet in these conditions vaccine therapy frequently fails to produce beneficial results, due, in all probability, as Wright believes, to the failure of the immunizing mechanism of the patient to produce antibodies.

In staphylococcal infections good results frequently will be secured by using a stock vaccine; yet it is preferable to use an autogenous preparation, which will in many cases prove efficacious when a stock vaccine has failed. So, wherever possible, cultures should be made and a vaccine prepared from the specific bacteria present in the individual case. In cases of acne vulgaris where an acne-bacillus vaccine is indicated the stock vaccine has proved to be equally as effective as the autogenous; and, owing to the difficulty in growing the acne bacilli, even by those specially prepared for the work, the stock preparations of acne bacilli are largely used.

In the vaccine treatment of cutaneous disorders certain important factors must be considered, such as the dosage, the interval between inoculations, the virulence of the infecting organism, and individual susceptibility. These will be discussed later.

In the preparation of any vaccine the bacterium which is the etiologi-
cal factor in the case must be isolated and grown in pure culture. In certain conditions, such as furunculosis and carbunculosis, this is a comparatively easy procedure, since there is usually but one causal organism in such disorders.

ACNE

In acne a somewhat different problem confronts us, since there are three organisms usually present in the comedo—a small bacillus, a coccus, and the bottle bacillus, or flask-shaped bacillus of Melassez—the two former being also present in the pustules. There is still some difference of opinion among investigators as to the exact relation each of the above-mentioned organisms has to acne lesions. The bottle bacillus, being more numerous in areas where acne lesions do not occur, may be disregarded as an etiological factor. The small bacillus and the coccus are the two organisms which have been studied carefully by various research workers.

In 1893 Unna (16) found a bacillus in the comedo which was present so constantly that he believed it to be the cause not only of the comedo, but also of the acne pustule. He called it the acne bacillus. Later he changed his opinion and considered it as only a saprophyte. Engman, who was working in Unna’s laboratory at that time, succeeded in growing the bacillus, but not in pure culture. Later, however, he was successful in his efforts to secure pure cultures (1). The following year, 1894, Hodara (9) published the results of his investigations, which were made on the comedo. His researches showed that the small bacillus was always found in the deeper part of the comedo, the coccus being more superficial. Unna had previously made the same observation. The coccus was thought by him to be a saprophyte and not a staphylococcus. His description of the bacillus was very similar to that of Unna. On account of the organism being so deeply seated, and the great difficulty encountered in growing it artificially, he concluded that it grew only under anaerobic conditions. He finally succeeded in getting cultures by immersing the comedo in alcohol for two or three days and then inoculating agar media. He also believed it to be the cause of both the comedo and the acne pustule.

In 1897 Sabouraud (12) described a bacillus which was morphologically identical with that described by Unna. He believed it to be the cause of an oily seborrhea which preceded the comedo and other lesions of acne. He succeeded in growing the organism in pure culture on an acid peptone-glycerin medium.

Gilchrist in 1899 (6) and 1903 (7) published the results of extensive cultural and inoculation experiments. He succeeded in isolating the microbacillus described by Sabouraud so frequently that he concluded the
presence and growth of the bacillus were the cause of the acne pustule, and that the staphylococcus albus was a secondary factor. Animal inoculations were made and the bacillus was obtained in pure culture from different organs. Agglutination tests were positive. He gave the bacillus the name of "bacillus acnes."

Fleming (3) believes that the comedo and pustule are caused by the acne bacillus. In ninety-seven per cent. of the pustular lesions examined he found the bacillus in film preparations. A staphylococcus was found with the bacillus in fifty-three per cent. of film preparations. By rubbing a pure culture on sterilized skin he produced pustules from which pure cultures of the bacillus acnes were secured. He also noted that the opsonic index in acne patients varies from the normal, and that an excessive dose of acne vaccine may produce a negative phase, accompanied by new pustules, from which the acne bacillus may be grown in pure culture.

Whitfield (19), in 1910, believed that the bacillus was the cause of the comedo, but not the pustule; the latter being due, in his opinion, to secondary infection with staphylo cocci.

In 1910 Engman (2) reviewed his previous work and experiments and concluded that the acne bacillus was not only the cause of comedones, but also pustules in some cases.

Trachseler (15) agrees with Unna's later opinion that a micrococcos is the essential etiological factor in both comedo and pustule. After isolating the organism it was transferred to the normal skin of three acne-free patients. Comedones developed from which the same organism was grown.

A diplococcus giving a white growth in culture was obtained by Varney and Clark (17). Morphologically it differed from staphylococcus albus. They secured the organisms from a number of different cases.

Recent experiments have proved that the biological characteristics of the varieties of a bacterial species are not fixed, but may change under both natural and artificial conditions, and hence it is not improbable that the different forms of cocci isolated from acne lesions are but different phases in the development of one organism. When the bacillus acnes can be grown artificially to such an extent and in such manner that cultures, subcultures, and inoculation experiments can be carried out more readily, future work will in all probability clear up the question of relationship between the different types of bacteria that have been described in connection with the etiology of acne.

**Acne Bacillus.**—The acne bacillus is a small organism, varying in length from one to four microns and about one half a micron in width. It is Gram-positive, and in smears is found either singly or in pairs, at times an end-to-end arrangement being seen. It shows a bipolar, darkly stained body. These darkly stained areas may present a chain appearance.
In smears from the acne lesions the microscopical characters are similar to those of the diphtheroid bacilli. It stains readily with the usual stains used in bacteriological work.

Cultural Characteristics.—As first suggested by Hodara, and later proved by Gilchrist (7) and others, the acne bacillus grows best under anaerobic conditions, the tubes being rendered anaerobic by Wright's or any other efficient laboratory method. In plain broth, after three or four days, small, grayish-white bodies are seen at the bottom of the tube. The staphylococci are in larger colonies and are more profuse during the first few days, but later cease to develop. On plain agar, two per cent. glucose agar, or one-fifth per cent. oleic-acid agar, the colonies, after three or four days' incubation, appear grayish-white in color, are slow in development and growth, and are very easily separated from the underlying medium. It is very difficult to grow the organism under aërobic conditions.

Preparation of Vaccine.—When cultures are made from the superficial pustules there is usually little difficulty encountered in securing a vigorous growth of staphylococci, as this organism is so frequently the cause of such lesions. When material is secured for purposes of inoculation from the deep nodular lesions or from subcutaneous abscesses which tunnel under the skin, in some instances forming lesions containing a dram or more of pustular material, frequently streaked with blood, and plainagar or blood-agar cultures made and incubated under aërobic conditions, as a rule no growth will develop, because it is in such type of lesions that the acne bacillus is the etiological factor. If cultures are made and placed under anaerobic conditions a growth will develop after three or four days in a considerable percentage of cases. The observations made by Unna and Hodara in regard to the bacillus being at the base of the comedo and deeply situated readily explain its relation to the type of abscesses above described.

Inoculations, both anaerobic and aërobic, are made from the superficial and deep pustular lesions, also from the comedones, on suitable media, as described above. If, after three or four days, no colonies develop another attempt should be made. If only one type of colony develops a study of the organism can be made at once for identification. If more than one bacterium is present further work is necessary in order to isolate each organism. After securing a pure culture by dilution and plating, preferably on solid medium, it is washed or rubbed from the surface of the medium with sterile normal salt solution. This suspension of living bacteria is then transferred to another receptacle and shaken vigorously to break up masses of bacteria. It may be necessary to filter the suspension through coarse filter paper in order to eliminate some of the larger clumps or masses of bacteria. With staphylococci a twenty- to twenty-four-hour culture should be used; with the acne bacilli a longer interval must elapse to get sufficient colonies for the vaccine on account of the slow growth and
development of this organism. After a uniform suspension of the living bacteria is secured the preparation is sterilized by heating in a water bath for one hour at 60° C. The receptacle containing the suspension must either be sealed and entirely immersed or the part above the water must be previously sterilized in a flame, in order that all bacteria may be killed. After heating the required period of time cultures are made, and if no growth develops the suspension is sterile and ready to be standardized. By standardization is meant the estimation of the number of bacteria in one cubic centimeter of the suspension. This is accomplished by one of two methods. One of these is by use of the hemocytometer, using the pipette ordinarily employed in making a red-cell count. Carbol fuchsin is used as a diluting agent, using one part of the stain to nine parts of water. This will stain the bacteria sufficiently to be easily counted. The count will be in terms of one cubic millimeter, from which the number in one cubic centimeter is readily computed. This method has been found to be entirely satisfactory in the hands of the writer. The other method of estimating the number of bacteria per cubic centimeter is to take equal parts of defibrinated blood and of bacterial suspension that has been diluted a definite number of times; a smear is then made on a slide, stained by one of the usual stains, and the number of red cells and bacteria counted in a number of fields. Placing the normal red count at 5,000,000 per cubic millimeter, the relative number of bacteria can easily be determined.

After the vaccine is standardized it can be diluted with normal salt solution to any extent desired. One-fourth per cent. phenol or tricresol is used as a preservative. The diluted vaccine can either be kept in a container or placed in 1 c. c. glass vials, one dose in each. These are sealed and kept in a cool place ready for use.

The Reaction after Inoculations.—The vaccine is given subcutaneously by means of an ordinary hypodermic syringe and needle, previously sterilized. The external surface of the upper arm is usually the site selected. Too many injections should not be given in the same area, as considerable induration may result. The local reaction depends to a large extent upon the size of the dose. A small dose is usually followed by very little discomfort. Some tenderness may develop six to twelve hours later. When larger doses are given there may be considerable tenderness, swelling, erythema, and induration within twenty-four hours after the injection. This is usually an indication that too large a dose has been given, although occasionally very small doses cause severe reactions in extremely susceptible patients.

Little, if any, systemic reaction follows an injection. The following is quoted from the report on vaccines by the committee appointed by the Council for Pharmacy and Chemistry of the American Medical Association:
The phenomena of anaphylactic reaction are encountered as a rare event in the course of a series of bacterial inoculations. This reaction may be localized at the site of a previous inoculation which becomes temporarily swollen and irritated. More marked hypersensibility is shown by erythematous or other skin eruptions, usually accompanied by itching. In still less frequent instances the acute angioneurotic symptoms of urticaria have been noted. There is no record of fatal anaphylactic shock following the inoculation of bacterial vaccines. Fox reports two cases treated with autogenous vaccine that developed alarming symptoms: Following the first injection there was high fever, headache, nausea, dizziness and severe local symptoms. Similar cases have been reported by Wallace and Hitchins.

After having given a large number of inoculations I have never seen a case show evidences of angioneurotic disturbance.

In the vaccine therapy of cutaneous diseases, as in other diseases, the opsonic index was used for a time as a guide to regulate the size and interval between doses. In the hands of skillful laboratory workers it is probably of some value. Wright was able to prove the different phases of reaction by means of the opsonic index. For instance, if the opsonic index fell below the previous level it was termed by him "the negative phase of immunizing response"; the subsequent rise above the previous level was denominated the "positive phase." By a careful adjustment of the size and frequency of dosage a condition is attained in which there is a very short negative phase followed by a long positive phase.

When acne-bacillus vaccine was first used too large doses were usually given, and as a result the injection was followed by new lesions, showing a lessened immunity on the part of the patient. When smaller doses are given this objectionable feature can largely be overcome.

Since so many varied opinions are held as to the etiological factor concerned in oily seborrhea, comedones, and acne pustules, the results from vaccine therapy are necessarily not entirely satisfactory at the present time. I have used it, however, in many cases, and find that it is a valuable addition to other methods of treating the different types of acne. The improvement in some cases is quite marked, while in others little or no benefit follows the use of vaccines. When they are administered in conjunction with other treatment, both local and general, beneficial results are seen in a sufficient number of cases to justify their use, especially in those cases that do not react readily to local treatment. In many cases deeply seated nodules and abscesses are absorbed and disappear with injections of acne-bacillus vaccine, the patients having very little local treatment during the period of inoculation. Other similar cases improve but little. Superficial pustules frequently disappear after injections of a staphylococcus vaccine; recurrences one or two months later are frequent, indicating either that the staphylococcus is only a secondary infection and that the primary cause is still present, or that the immunity by inoculation with staphylococci is of a temporary character.
TREATMENT. — The method of procedure depends entirely upon the predominating type of lesion. There are three groups of cases: (1) those in which superficial pustules due to staphylococci predominate; (2) cases presenting many deep-seated nodules and abscesses, in the development of which the acne bacilli undoubtedly are an important factor; and (3) those cases in which the comedones are the chief source of concern on the part of the patient. Some cases present all three types of lesions.

In the first group a stock, or preferably an autogenous, vaccine, made in the manner described, is used. At the first inoculation 100,000,000 staphylococci should be injected. If there is but little local reaction at the end of twenty-four hours, and few, if any, new lesions develop in the following three days, another injection of 200,000,000 bacteria should be given five or six days following the first. Then, at intervals of five or six days, injections are given, gradually increasing the dosage to 500,000,000 bacteria. If, when larger dosage is given, a markedly negative phase is observed, manifested by many new lesions three or four days after injection, the number of bacteria must be decreased.

If a few deep-seated nodular lesions coexist with the superficial pustular type of lesion from 3- to 5,000,000 acne bacilli can be administered, either with or separately from the staphylococcus vaccine.

There is no rule that can be made in regard to dosage that will apply to all cases; it depends not only on the nature of the infection, but also on the individual susceptibility. A prolonged negative phase, lasting for five or six days, indicates an excessive dose; a short negative phase, followed by a short positive phase, indicates too small dosage. The local reaction following the injection must also be interpreted properly. Marked tenderness, with induration and erythema, prolonged for several days, is an indication of excessive dosage.

In the second group of cases, or those where the acne bacilli are present as the most important etiological factor, Engman's technique in administering the vaccine has proved to be of value. However, I have failed to observe the brilliant results secured by him. Three million acne bacilli are given at the first injection. If over three or four new lesions appear in the following three days the dose is too large. After three days the lesions are opened, gently massaged, the larger comedones extracted, and moist hot compresses applied twice each day. On the sixth or seventh day a second injection of from 3- to 5,000,000 bacteria is given and the same local treatment repeated for three days. After this the dosage is gradually increased to 10,000,000. Small doses at intervals of from five to seven days have given the best results.

In some cases larger doses may be tolerated by the patient and are indicated. Great care must be exercised in giving increased dosage, especially as to the development and number of new lesions following such an injection.
Patients in whom many superficial pustules are present, together with the deeper type of lesions, may require a combination of vaccines of the staphyloccocus and acne bacillus.

Gilchrist (5) did not have very marked success with Engman’s method, and has returned to his former plan of giving larger doses at longer intervals. He recommends an initial dose of from 10- to 20,000,000 of acne bacilli. The negative phase, which occurs from the third to the eighth day with such a dosage, is manifested by the appearance of new lesions and increased activity of the old ones. When these begin to subside another injection is given, the same procedure being repeated three or four times, followed by a period of rest. Improvement results usually in from four to six weeks. When relapses occur two or three injections are given at weekly intervals.

Vaccine therapy in the third group of cases, in which the comedo is the predominating lesion, has not proved to be a very important addition to other methods of treatment. In excessively oily seborrhoeas associated with comedones a vaccine made from Sabouraud’s microbacillus has been used with variable results.

Bab (1) reported 35 cases of acne treated with vaccines. His initial dose was 50,000,000 staphyloccoci, increased to 300,000,000, and in some cases to 500,000,000. If autogenous vaccines were used, the dosage was smaller. He used the vaccine in selected cases, in which there was considerable suppuration. The appearance of the lesions improved and there was less suppuration, but the improvement was not permanent. Even autogenous vaccines did not effect a cure. However, he concluded that vaccine treatment combined with or preceding Roentgen rays was useful, and recommended this combination of therapeutic measures for severe cases.

Morris and Doré (11), after an extensive use of vaccines in acne, do not recommend their use as a routine treatment, but prefer to reserve them for carefully selected cases. These selected cases are divided into three groups: The first consists of cases characterized by deep-seated pustules, with considerable induration, situated on the face, chest, and back; in which staphyloccoci predominate. In such cases a staphyloccoeic vaccine, administered during a period of several months, is of some value. The second group comprises cases in which the lesions are superficial and indolent, mostly papules, with very few pustules and comedones. In these cases an acne bacillus vaccine gives good results in a large proportion of cases. In the third group are placed cases which overlap the first and second groups, cases in which both very active and indolent lesions are present. In these a mixed staphyloccocus and acne bacillus vaccine may be used with fair results in many cases.

The importance of general measures is emphasized in the management of every case. They conclude their report as follows: "Experience
FURUNCULOSIS

prevents us from claiming more for vaccine therapy in acne than that it is a useful adjunct of the ordinary forms of treatment."

Smith (14) reported 150 cases of acne which he had under observation. He secured the most favorable results in cases having deep-seated pustules due to the staphylococcus albus; the dosage was 10,000,000 staphylococci. Less satisfactory results were secured when the papules and pustules were superficial, and when both acne bacilli and staphylococci were present. In such cases, 5,000,000 acne bacilli and 10,000,000 staphylococci were given at each injection.

Lovejoy (10), in 1912, reported 50 cases of acne, characterized by indurated and pustular lesions, treated with vaccines. Some of the cases received stock staphylococccic vaccine only, while others received an autogenous vaccine composed of 3,000,000 acne bacilli and 150,000,000 staphylococci, at five-day intervals; the dosage being gradually increased to 5,000,000 acne bacilli and 250,000 staphylococci. All the cases showed marked improvement; the results were as satisfactory with stock vaccines as with autogenous preparations.

In 100 cases of acne treated by autogenous vaccines, Smiley (13) reported 93 cases cured and the remaining 7 cases improved. He used an initial dose of 150,000,000 staphylococci and 5,000,000 acne bacilli. These were given on the same day, but separately. The dosage was controlled by the length and degree of the negative phase. Injections were given every second day. Fox (4) recently reported one hundred cases of acne and furunculosis treated with vaccines. The acne cases were classified in two groups, those having "coarse" and more deeply seated lesions being placed in the first group. The second group comprised those with "fine" or more superficial papules and pustules. Both autogenous and stock preparations of vaccine were used; the dosage is not given. Three months after treatment with autogenous vaccines, no patient was free from lesions; 25 per cent. of the cases showed improvement. With stock vaccines, 18 per cent. showed improvement three months after the last treatment. During the course of the treatment a much larger percentage showed improvement.

In the furunculosis cases the percentage of cured and improved was the same, in those treated locally and in those treated both locally and with vaccines.

FURUNCULOSIS

In the entire list of disorders due to bacterial infection there is probably none which offers more evidence in favor of vaccine therapy than furunculosis. It was in this condition that Wright first tried inoculations with staphylococci, and in which he secured favorable results. There are some cases, however, of furunculosis that do not respond readily to vaccine treatment, regardless of the nature of the vaccine, whether it be a stock or
an autogenous preparation. The failure to get results may be due, in some instances to errors in the size or interval of the inoculations.

Whenever possible, it is advisable to use an autogenous vaccine in furunculosis. While a stock vaccine proves beneficial in a certain percentage of cases, the improvement is more rapid and occurs in a greater number of patients if an autogenous preparation is used.

Usually the staphylococcus aureus is the organism isolated, although I have seen cases in which both albus and aureus were present, and some in which the staphylococcus albus alone was found.

If it is impossible to have an autogenous vaccine made a stock preparation of staphylococcus aureus may be employed with a reasonable hope of benefit.

**Preparation and Dose of Vaccine.**—Cultures should be made on either plain or glycerin agar from recent lesions, from which no pus has escaped. An eighteen- or twenty-four-hour growth is best suited for the vaccine. If care is exercised in the selection of lesions for cultures, in the majority of instances a pure culture is secured at the first attempt. The vaccine is prepared in a manner similar to that described under the treatment of acne.

As in other conditions amenable to vaccine therapy, the question of dosage and interval between injections is of the utmost importance. Although no set rule can be given in regard to the administering of the vaccine, a safe procedure is to begin with rather small dosage—50- to 100,000,000 in adults, about 20,000,000 for children, and 10,000,000 for infants—at five-day intervals. Using the local reaction and clinical course of the disease as a guide, the dosage may be increased until 500- to 1,000,-000,000 are given to an adult at one injection, 100- to 200,000,000 to children, and from 50- to 75,000,000 to infants. Frequently, after two or three injections, no new lesions will appear and the pre-existing ones gradually clear up. Three or four inoculations should be made after the disappearance of all lesions.

If there should be a recurrence of the furuncles after an interval of several weeks or months, the vaccine should again be given and be continued for a longer period than previously after the disappearance of all lesions. When the vaccine is administered properly there is much less probability of a recurrence than with any other method of treatment.

**Sycosis Vulgaris**

This type of sycosis is usually due to the staphylococcus aureus, and is frequently quite resistant to local treatment, owing to the deep-seated character of the infection. When an autogenous vaccine can be prepared, it should be used, for in many cases it will aid very materially in the treat-
ment of this disorder. The dosage should begin with 100,000,000 bacteria and gradually increase to 500,000,000 at intervals of five or six days. Stock vaccine will prove of value in some cases.

**CARBUNCULOSIS**

Carbuncles are frequently preceded by malaise, chill, and pyrexia of varying degree. In cases in which successive carbuncles appear in or near the same area vaccines are of material assistance in the management. Owing to the deep localization of the lesions, local applications do not come readily in contact with the source of the disease. Surgical measures should not be delayed in any severe case merely to see what benefit may be derived from the vaccines.

The technique of administering the vaccines is similar to that in furunculosis. As in other conditions, autogenous vaccines are preferable.

**ECZEMA**

In certain cases of chronic eczema associated with a pustular infection, and more or less weeping, vaccines have given favorable results. In the dry, scaling type no benefit has resulted. In eczema seborrhoeicum a number of different organisms have been found. In one case the writer isolated a coccus that corresponded to Unna's micrococcus. A vaccine prepared from this organism was of doubtful value.

**ROSACEA**

In rosacea of the pustular type autogenous vaccines will cause the disappearance of the pustules, and as the inflammation subsides the erythema becomes much less prominent. Gilchrist (5) reported fifty cases with very satisfactory results. He prefers giving rather large dosage at longer intervals both in acne rosacea and acne vulgaris.

**OTHER DERMATOSES**

Vaccines have been tried in other dermatoses of both known and unknown origin, such as the different types of pemphigus, mycosis fungoides, favus and ringworm, dermatitis herpetiformis, the different forms of erythema multiforme, chronic urticaria, pityriasis rosea, psoriasis, dysidrosis, lichen planus, and other conditions, but the results, as a rule, have not been encouraging.
REFERENCES

15. Trachsler. Dermat Studien (Unna Festchrift), 1911, 1311.
CHAPTER XXIII

INFECTIONS OF THE MOUTH. INFECTIONS BY FUSIFORM BACILLI AND SPIROCHETES. ACUTE ULCEROUS GINGIVITIS. ERY-SIPELAS—INFECTIONS OF SINUSES—OTITIS MEDIA. SECONDARY INFECTIONS. MIXED VACCINES

ERNST E. IRONS

INFECTIONS OF THE MOUTH

INFECTIONS BY FUSIFORM BACILLI AND SPIROCHETES

In the normal mouth may be found a great variety of bacteria and other organisms, many of which are continually introduced from without through food and air. The relative immunity of the tissues of the mouth to infections by this multitude of organisms was formerly ascribed to a supposed antiseptic property of the saliva, but more recent researches have shown that this property is very slight, if present at all, and that the freedom from infection is referable rather to the abundant blood supply which the structures of the mouth share with other tissues of the face and head. It is also a notable fact that when infections occur, conditions of relatively decreased oxygen supply are often present in the lesions.

In addition to the commoner bacteria which occur in the mouth and tonsils, certain spirochetes (S. dentium, S. buccalis), entamebæ, and protozoa have been described in the normal mouth. (Küster, 2.)

Infections about the teeth, such as pyorrhcea and alveolar abscess, have recently received attention through the recognition that such lesions may be the source of infectious processes manifested by arthritis and allied conditions, as outlined in Chapter IV on Focal Infections.

The etiologic relation of pyorrhcea to arthritis and other metastatic infectious lesions seems clear in certain cases, but it must be remembered that there are many cases of pyorrhcea in which there is no evidence of arthritis, and still others in which pyorrhcea may be merely coincident with another focus of infection which is the real source of the metastatic lesions. The relation of alveolar abscess to metastatic processes, including
arthritis, has been clearly demonstrated in a number of cases. Gilmer and Moody have investigated the bacteriology of a large number of infections of the alveolar processes, and have found that the infecting organisms are usually streptococci and anaerobic organisms such as spirochetes and fusiform bacilli.

Weaver and Tunnicliff (3) and others have cultivated fusiform bacilli from the lesions of Vincent's angina and from noma. Fusiform bacilli may give rise to fatal septicemia, as in the case studied by Rosenow and Tunnicliff (4). Cases of infection apparently originating about the teeth and alveolar processes may extend into deeper tissues of the jaw and neck, producing at times an enormous brawny induration, associated with fever, chills, and other signs of general infection. From the scanty pus obtained from a few of these cases organisms morphologically like fusiform bacilli have been cultivated.

**Acute Ulcerous Gingivitis**

In 1906 Gilmer (1) described a disease of the gums to which he gave the name acute ulcerous gingivitis, and outlined the clinical picture as follows: The onset of the disease is sudden, the earliest symptoms indicated by a slight malaise, which is quickly followed by rapid ulceration, at first confined to the gingiva, usually about two or three of the anterior teeth on both jaws simultaneously, and in corresponding localities; later it is extended to the gums about a number of teeth, or groups of teeth, but rarely, if ever, does it include the entire gum margin. The lingual margins and festoons of the gums do not participate at first in the inflammatory processes, but later the festoons are destroyed and deep pockets are formed in the interproximal spaces. Still later the lingual gingiva participate, but in no case have I seen ulcerous manifestations in this locality.

In 24 hours after the patient's attention has been called to the condition of his gums, the parts attacked present the appearance of having been gnawed away until most of the gum tissue overlying the alveolar process immediately adjoining the teeth has been destroyed. The part of the gum attacked has soft, thickened, and in some instances everted margins. The eroded parts form pockets, which are filled in with a grayish pasty mass, similar to that found in syphilitic ulcers in the mouth. When this mass is removed a granulating surface is exposed, which bleeds easily and is very painful to the touch. The mucosa for a short distance from the ulcerative margins is of a dark red hue as a result of congestion. The gum covering the cervical and interproximal surfaces of the teeth is destroyed sufficiently in some cases to uncover the teeth down to the alveolar border.

More recently Gilmer and Tunnicliff have demonstrated fusiform bacilli in the cultures from the depths of these lesions.

In two cases of this disease observed in children two years of age by
ERYSIPELAS

F. B. Moorehead and the writer, in addition to the progressive necrosis of the alveolar process, there were symptoms of general sepsis with high fever, sometimes continued, sometimes intermittent, with sweating, and loss of weight, together with a peculiar recurrent multiform rash, occurring on the trunk, but also to a less extent on the extremities, at first macular, dark red, or bluish, with hemorrhagic puncta later becoming scaling. The disease ran an irregular downward course with brief periods of temporary improvement. One child died about six months after the onset. Syphilis was excluded with reasonable certainty.

Specific Treatment of Dental Lesions

Pyorrhea and Alveolar Abscess.—The treatment of pyorrhea by the injection of vaccines is justified neither by anatomic and pathologic considerations nor by clinical results. Alveolar abscess calls for surgical treatment, and when this is afforded either by extraction or by other approved dental procedure, the lesion heals without the use of vaccines.

Infections by B. Fusiformis.—A limited number of infections by fusiform bacilli have received autogenous vaccine treatment; in several the results have been said to be good, in others, including one case of ulcerous gingivitis, no benefit was observable. In ulcerous gingivitis Gilmer advises calomel internally combined with local application of hydrogen peroxid.

ERYSIPELAS

The course of erysipelas is so variable and erratic that the determination of the value of any method of treatment presents great difficulties. As might be anticipated, the demonstration of any effect of vaccines on the course of the disease is especially difficult, and opinions differ greatly as to whether the duration of the infection is modified at all. Recurrences have been noted as less frequent in treated cases by several workers who were unable to see any limitation by vaccines of the attack during which inoculations were begun. In the cases with a minimal degree of constitutional disturbance, and with a slowly migrating type of infection, vaccines may be used during the attack and as a prophylactic against recurrences, but a successful outcome of the method cannot be regularly attained. Thus Weaver and Boughton (8) were unable to see any clear benefit from vaccines in 22 acute cases. In three migratory cases the advance of the disease ceased after vaccines. In a chronic recurrent case studied later by Boughton prolonged vaccine treatment had no effect in stopping the course of the disease. Erdman (5) has recently made an interesting analysis of the clinical course of 800 cases of erysipelas under
his care in Bellevue Hospital during seven periods of service aggregating forty-two weeks from 1909 to 1913.

**Table Compiled from Erdman's Series of Cases of Erysipelas**

<table>
<thead>
<tr>
<th>Type</th>
<th>Untreated Cases</th>
<th>Treated by Vaccines</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Duration (Average Febrile Period in Days)</td>
</tr>
<tr>
<td>Uncomplicated facial</td>
<td>500</td>
<td>6.77</td>
</tr>
<tr>
<td>General body and migratory</td>
<td>56</td>
<td>14.44</td>
</tr>
<tr>
<td>Leg cases</td>
<td>33</td>
<td>10.88</td>
</tr>
<tr>
<td>Combined mortality of entire series</td>
<td>800</td>
<td></td>
</tr>
</tbody>
</table>

Two hundred and eleven cases were complicated with other diseases such as septicemia, extensive cellulitis, mastoiditis, empyema, tuberculosis, rheumatism, pneumonia, acute alcoholism, and were so variously influenced by the complication that an average duration loses significance. In these cases, which are not included in the above table, the average duration was about 14 days, and the mortality 13.7 per cent.

The methods of use of vaccines as to dosage and interval of inoculation conformed to those usually recommended by other workers. The following extracts from Erdman’s paper are of interest in relation to the effects observed from vaccines compared with those in uninoculated cases:

"Immunity.—Four patients had recurrent attacks or erysipelas within from two to ten days after the attack, during which they had been given from five to seven increasing doses of vaccine.

Limitation of Lesion.—In cases in which there was localized facial erysipelas on admission, in which vaccine treatment was then instituted, at least seven developed the migratory form of the disease which spread down over the body.

Effect on Symptoms.—No constant or significant effects were to be noticed either in the temperature curve or in the symptoms of the disease after these injections, and such irregularities as occurred on the charts were to be found on many charts of untreated cases which were in the wards at the same time.

Complications.—Cellulitis and abscesses occurred in some of the vaccine cases as in those not so treated.

From our experience with vaccines in erysipelas I must state that the duration of the disease was not at all lessened, the mortality remained at the same level, and there was no immunity guaranteed against recurrence, against spreading of the lesion, nor were complications, such as cellulitis
and abscesses, prevented; from the statements furnished by the patients, moreover, I could not gather that there was any amelioration of the subjective symptoms."

Hiss (6) has used leukocytic extracts obtained by the digestion of the cellular intrapleural exudate in rabbits following aleuronat injections in the treatment of erysipelas in man, and believes that the symptoms of the disease are ameliorated and the course shortened.

**INFECTIONS OF SINUSES—OTITIS MEDIA**

The inoculation of vaccines in chronic suppurrative processes of the ear and of the accessory sinuses of the head has been extensively practiced. Weston and Kolmer treated 100 cases of otitis media with vaccines, comparing the duration under treatment with that of 200 cases in which vaccines were not used. In both groups about 15 per cent. failed to heal, but in those which received vaccines the healing was more rapid. In general it may be stated that the vaccines have added little, if anything, of proved value in the treatment of infections of the ear and sinuses. Such lesions call for appropriate surgical or other treatment which shall secure adequate drainage and the removal of sources of mechanical irritation.

**UNWARRANTED USE OF MIXED VACCINES**

Active immunization has been brought into grave disrepute by attempts to utilize vaccines as a cure-all in diseases in which there is no evidence that they are of value, in those of undetermined infectious origin, and in those in which there is no evident infectious cause. The literature on vaccine and serum therapy is loaded with favorable reports of such procedures, based on mistaken premises and supported by conclusions entirely unwarranted by clinical facts. In consideration of the scientific basis of active immunization this misuse of vaccines is reprehensible and deplorable; injurious to the unfortunate patient and demoralizing to the physician.

The absurdity manifested in the treatment of diseases of unknown infectious etiology and of those in which there is no evidence of infectious origin by commercial mixed vaccines is surpassed only by the inoculation into patients suffering from various diseases, infectious and otherwise, of filtrates containing the metabolic products of a variety of pathogenic bacteria.

Such a procedure is indefensible on scientific grounds, and is not supported by adequate clinical facts. Pearce (7) expresses the views of both the clinician and the immunologist as follows:

"That many physicians claim excellent results for vaccines in the treat-
ment of diseases of obscure origin is not conclusive. It is in just this
class of diseases that the osteopath, the naturopath, and others of the
same group likewise claim most brilliant results for their peculiar
methods.

Who is to judge of the influence of a single factor in the treatment
of disease?

Has not past experience shown that early results with new cures are
usually brilliant?

How many diseases heal spontaneously or are improved despite any
treatment?

How important is the psychic influence of a new widely heralded
treatment? And, on the other hand, are the unsuccessful results always
reported; do we learn, except by accident, of the failure to improve, and
of sudden death due to the treatment?

Many factors must be considered in arriving at an opinion.

The present attitude with regard to vaccination in diseases of doubt-
ful origin promises little for either etiologic diagnosis or specific treat-
ment, the two aims of modern medicine, nor does it aid that broader ef-
fort to advance social welfare, in the furtherance of which medicine, if
it is to remain a powerful factor, must be both safe and sane.

If the venturesome practitioner feels compelled to use vaccines in
diseases of obscure origin, let him do so supported by every aid which
laboratory and clinical research may offer; but the wiser course, in the
absence of a demonstrable local infection from which an autogenous vac-
cine may be made, is to leave vaccination to others. One fact, the essential
mechanism of vaccination, must not be misinterpreted or minimized, and
this is especially important in view of the advertised statement concern-
ing commercial vaccines. The statement that ‘the growth of infecting
microorganisms can be arrested and their effects neutralized by the
products derived from their development in artificial culture mediums’
is misleading in that it implies direct bactericidal and antitoxic action
—which is not the case—and leaves out of the question the only ra-
tional explanation of vaccination, that it is the stimulation of the im-
munizing process in the host. This misleading statement of the com-
mercial vaccine-maker is responsible for much of the misunderstanding
concerning vaccine therapy and its curative value.

Mixed vaccines, commercially prepared, constitute a type of bacterial
polypharmacy which should be discouraged as unscientific and unethi-
cal.

Therapeutic vaccination, if it is to be placed on a scientific basis,
should be regarded as a method of treatment based on the study of the
individual and his infection, and not as a ready-made method capable
of the universal application of stock vaccines. The use of vaccines in
diseases of unknown etiology is unscientific and ethically indefensible.”
REFERENCES

Infections of the Mouth


Erysipelas

CHAPTER XXIV

SPECIFIC REMEDIES IN SCARLET FEVER

G. H. Weaver

Numerous investigators have attempted to discover a specific cause for scarlet fever, and while various bacteria and other microscopic bodies have been described as characteristic of the disease, none of them has stood the test of study by later students of the subject. Investigation of the etiology, and consequently of the cure, of scarlet fever has been especially difficult, because, like most of the human contagious diseases, it cannot be successfully transmitted to lower animals, with the possible exception of certain apes which have been practically excluded from the experimental work because of their cost. The frequent association of secondary infections has also served to render these studies more difficult. It is quite generally agreed that the cause of most of the secondary complications of scarlet fever, as of those of most of the contagious diseases, is a streptococcus. So constant is this association of streptococci with the various lesions of scarlet fever that some students of the disease have insisted that a peculiar form of streptococcus is the essential cause of scarlet fever. Most of those, however, who have studied this problem have concluded that streptococci are only secondary factors, and, while responsible for most of the fatality in scarlet fever, still are entirely distinct from the real cause of the disease. Acting upon the assumption that during the course of scarlet fever specific antibodies are formed and accumulate in the blood of the person affected by the disease, various investigators have removed blood from persons who have just passed through an acute attack of the disease, and have injected the serum obtained therefrom into persons in the acute stage of scarlet fever, thus hoping to produce a passive immunity to the infecting agent and so aid in recovery. In 1897 Weisbecker (19) treated various infectious diseases by injections of serum obtained from convalescent patients who had just passed through an attack of the corresponding disease. Among the patients thus treated were five with scarlet fever. His results were not very encouraging. About the same time Huber and Blumenthal (5) reported the results of the treatment of thirteen cases of scarlet fever by subcutaneous injections of from 20 to 40
SPECIFIC REMEDIES IN SCARLET FEVER

c. c. of serum obtained from scarlet fever convalescents four to twenty-one days after the disappearance of fever. In 1902 von Leyden (7) added three cases to this series from the same clinic. These writers were not able to arrive at any definite conclusion as regards the value of the procedure, but as it was harmless advised further trials. In 1903 Rumpel (11) reported a series of 39 cases of scarlet fever in which subcutaneous injections of about 20 c. c. of convalescent scarlet fever serum were administered. He observed no effect on the complications, but thought there was some beneficial result in uncomplicated cases if the serum were given on the first three days of the disease. He noticed no influence on the fever, nor any shortening of the disease. About the same time Scholz (13) added his experience in nine additional cases, most of them mild, in which convalescent serum was administered, but he found any desirable therapeutic effect lacking.

Discouraged by the meager returns, and also deterred by the fear of transferring syphilis in the serum, this method of treatment was abandoned for nearly ten years. By this time it had become possible to exclude syphilis in the donor of the serum by the Wassermann test, and greater confidence had been acquired in the safety of administering larger amounts of serums in general. In 1912 Reiss and Jungmann (9) again instituted the treatment of scarlet fever by injections of serum from convalescent cases. They have departed from the course followed by the earlier authors by making the injections directly into a vein instead of beneath the skin, and by increasing very considerably the size of the dose. From 100 to 200 c. c. of blood is drawn from scarlet fever convalescents at the end of the third or beginning of the fourth week of the disease, the serum separated by centrifugation and 5 drops of a 5 per cent. solution of carbolic acid added to each 50 c. c. Each serum is tested for sterility and by Wassermann's reaction to insure the absence of syphilis. Several serums are then mixed, and portions suitable for single cases are sealed up in glass bulbs. They insist upon the importance of mixing several serums, since that yielded by some patients seems to contain more antibodies than that yielded by others. They treated with such serum 12 cases of severe scarlet fever. The serum was injected directly into a vein in doses of from 40 to 100 c. c., according to the age of the patient. In two cases no benefit was noticed. In ten the injection was followed by marked changes for the better. In from two to four hours the temperature began to fall and the pulse to slow down in proportion, and with this the nervous symptoms abated. The serum seemed to exert no influence over the lesions due to secondary infections. Still later Koch (6) has reported from the same clinic 22 additional cases, of which only one died. In some cases a chill and collapse associated with a rise of fever followed the injection. In general the serum appeared to exert a favorable influence over the disease, but complications such as otitis, arthritis, etc., occurred as in untreated
cases. In none of the treated cases did a hemorrhagic nephritis occur. Koch emphasizes the marked improvement in the general condition of the patient after the injection, this often being more pronounced than the fall in temperature. While there was no direct effect upon the lesions due to secondary infections, he noted that anginas appear to clear up very rapidly after the serum. Further use of such sera must determine whether we possess in it a valuable agent in the treatment of severe scarlet fever.

Some of the workers who have considered the streptococci associated with scarlet fever as the essential cause of the disease have undertaken to prevent it by means of streptococcus vaccines. Prophylactic injections of devitalized streptococci which have been cultivated from cases of scarlet fever have been employed by various Russian physicians. This form of procedure was introduced by Gabritschewsky (4) in 1905, and has been practiced extensively by several of his countrymen. The vaccine consists of a bouillon culture of streptococci isolated from persons sick with scarlet fever, which is heated to 60° C. and has 0.5 per cent. of carbolic acid added to it. The injections are given subcutaneously in the abdomen, thigh, back, or arm, the initial dose being 0.5 c. c. of the concentrated bouillon culture in children two to ten years of age, younger children receiving one-half of, and adults twice, this amount. Three injections are given at intervals of from seven to ten days, the size of each subsequent dose being increased one and one-half to two times. In most cases there appears at the site of the primary injection, about twenty-four hours after it is given, an area of redness and infiltration, somewhat painful and tender, and lasting a few days. With this is usually associated a slight rise in temperature. In about 10 to 15 per cent. of the cases an erythematous eruption resembling scarlet fever appears at the site of injection, and sometimes extends over most of the body, in which case there may be associated angina, swelling of the lymph glands, and "strawberry tongue." In a few cases there are high fever, slight albuminuria, and severe prostration. The reactions after the second and third injections are less severe. The injections are contraindicated in very young infants or in persons greatly prostrated from any cause, and in those with nephritis.

Smith (14), of Boston, has written a résumé of the most important publications upon this subject, many of which were originally printed in Russian. The statistics furnished by these Russian workers appear to speak very strongly in favor of the value of the prophylactic vaccinations. Watters (15) has reported the use of polyvalent vaccines in 21 nurses who had not been immunized by a previous attack of scarlet fever. The injections were given just before the nurse went on duty in scarlet-fever wards. Of the 21, one contracted scarlet fever, while of 14 nurses who were not given the injections 5 contracted the disease. These figures are, of course, too small to have other than suggestive value. The rather frequent development of symptoms resembling scarlet fever after the
injections of the vaccine as described by the Russian physicians causes one to suspect that the essential cause of the disease is found in their vaccine, or that many cases are about to develop the disease at the time of injection. Aside from those who have considered streptococci as the cause of scarlet fever, some who believe them to be secondary invaders have sought to attack them in a specific way through an active immunity brought about by injections of devitalized streptococci. Streptococcus vaccines prepared from organisms cultivated from cases of scarlet fever have been used both for the purpose of immunizing the patient during the early stages of the disease against subsequent complicating secondary infections and with the object of curing a streptococcus infection already present. In general it may be said that the use of streptococcus vaccines in cases of scarlet fever has led to disappointment. Little value has attached to them aside from that observed in connection with the subacute and chronic localized infections which persist after the acute stages of the disease have passed. It was hardly to be expected that the vaccines would accomplish much during the acute stage of a streptococcus complication when the protective forces of the body are already overtaxed in an effort to overcome the infection. Weaver and Tunnicliff (17) showed that streptococci killed by heat have lost most of their antigenic power, and Schenk (12) has recently decided after an experimental study that killed streptococci have little influence in bringing about streptococcus immunity. Producers of antistreptococcus serum in horses have insisted that living cultures are required if large quantities of antibodies are to be produced. The antigenic properties are better preserved if the bacteria are killed by other means than heat. Weaver and Tunnicliff (17) found that streptococci, devitalized by suspension in a strong solution of galactose, when injected into animals render them immune to living streptococci. This immunity is accompanied by an increase of streptococcus opsonin in the blood. Similar opsonic increase was found to follow the injection of such vaccines in man, and they observed the rapid healing of some subacute and chronic infections of the middle ear and nose following scarlet fever in patients given autogenous vaccines prepared in this manner. The dose of the galactose-killed streptococci is from 50- to 100,000,000 for the primary injection. Subsequent injections may consist of as many as 500,000,000, and the interval between injections should usually be from five to seven days. Too large doses and too often repeated ones are sometimes harmful. Weaver and Boughton (16) failed to reduce the later streptococcus complications of scarlet fever by administering vaccines early in the disease.

Antistreptococcus serums have been extensively used in scarlet fever. The serums used have generally been prepared by injecting horses with strains of streptococci obtained from cases of this disease. Some have used the serum with the expectation of curing or influencing the scarlet
fever, believing the disease to be due to streptococci, but most have used it to combat what they consider a secondary infection.

Baginsky (2), of Berlin, was one of the practitioners to use antistreptococcus serum in a large series of carefully studied cases. He was favorably impressed with the early results, but later abandoned the treatment because of the relatively slight value of the serum, and because of the danger to the patient from the toxic effects of the foreign serum when superimposed upon the already prostrated patient. The greatest advocate of antistreptococcus serum has been Moser (8), of Vienna. The serum employed by him is obtained from horses which have been injected with streptococci grown directly from fatal cases of scarlet fever, the organisms being secured post mortem from the heart's blood. The serum is injected in very large doses, and serum reactions are very frequent. Many favorable reports have appeared from those who have used Moser's serum. On the contrary, unfavorable reports are not lacking. For example, Axenow (1) reports the use of serum in 1,200 cases, in 683 of which, or 57 per cent., serum sickness followed. In 21 cases he ascribes death to the serum reaction alone. He also believes the serum increases the severity of the complications. He would reserve the serum treatment for the severest cases. Weaver and Tunnicliff (18) injected antstreptococcus serum in two cases of septic scarlet fever, and studied the effect upon the opsonin and the phagocytic activity of the leukocytes. Each case received 60 c. c. of serum intramuscularly. In each case a rapid rise in the opsonic index and in the phagocytic power of the leukocytes followed the injection of serum, and coincidentally the temperature fell and the leukocyte count decreased. Such observations impress one strongly with the power of the serum. It is difficult to judge the value of the treatment. Some men who at first were favorably impressed by the results following injections of the serum have subsequently abandoned its use. Very pronounced improvement sometimes occurs after the serum is administered, and a very severe or apparently hopeless case begins at once to improve rapidly. Such instances naturally make a favorable impression upon the observer, and he must be on his guard not to overlook the lack of effect in other cases. The favorable cases must always be compared with those in which no good effects are noted and with those which receive no serum at all. The opinion has become general that large doses of antstreptococcus serum are necessary to produce results, 30 to 100 or 200 c. c. being a proper dose. Severe serum reactions are more liable to occur with such doses. The fear that such reactions may sometimes more than counterbalance any benefit has led most clinicians of large experience in these cases to restrict the use of the serum to cases of the septic type coming under treatment in the early stages.

The secondary infections which persist after scarlet fever, such as otitis and rhinitis, have been treated by vaccines prepared from the special
strains of bacteria which have been cultivated from the lesions. Many such cases seem to be favorably influenced, but some are refractory. It should be remembered that such lesions tend to heal spontaneously about the sixth week, at which time the body is approaching normal health and nutrition. Vaccines do not act favorably in sinus and middle ear infections where free drainage is absent. Fischer (3) has injected neo-salvarsan into 15 cases of scarlet fever. The dose was usually 0.2 gm., but in a few cases was 0.3 or 0.4 gm. dissolved in 20 to 40 c. c. of sterile water. The injections were made intravenously. Septic cases were chosen. No deleterious symptoms followed the use in any case. The treatment rests on no reasonable basis, since there is no evidence presented to show that the drug employed has any effect upon streptococci, which are the harmful factor in the septic cases. Ross (10) has advocated the use of arsenic and mercury in cases of scarlet fever upon the theory that the disease is due to animal parasites which appear during the early portion of the disease as cell-inclusions in the lymphocytes. He would keep the patient under the influence of the drugs until ready to be discharged. He advances this as a measure to be tried, but offers no opinion as to its value.

REFERENCES


CHAPTER XXV

ANTISTREPTOCOCCUS SERUM

G. H. WEaver

The elimination of infecting streptococci from the animal body is accomplished naturally through the cooperation of several factors. The bacteria, or the products elaborated by them, or perhaps set free in their disintegration, stimulate the cells of the body to produce a substance known as immune opsonin which appears in the blood. This acts upon the infecting streptococci in such a way that they become phagocytosable by the leukocytes which then take them up. Within the leukocytes the bacteria are killed and disappear. The specific measures which have been employed to prevent and cure streptococcus infections have followed the lines indicated by nature in accomplishing these ends, i.e., they have acted by stimulating the production of immune opsonin in the body, or by introducing it ready formed. The production of immune opsonin is accomplished by the injection of what are known as vaccines. The vaccines consist of dead bacteria, and when injected in suitable quantities they stimulate the cells of the body to produce immune opsonins which correspond to the bacteria introduced. Vaccines act in a similar manner to living bacteria in an infection which terminates in recovery, and have the advantage that the toxic action can be accurately controlled. By repeated injections of streptococcus vaccines a considerable amount of opsonin results and appears in the blood. If a suitable healthy animal is given repeated injections of killed streptococci in increasing doses a large amount of streptococcus opsonin may finally accumulate in the blood. If the blood from this animal is drawn, allowed to coagulate, and the serum to separate, such a serum is what is known as antistreptococcus serum. Antistreptococcus serum contains various antistreptococcus bodies, such as agglutinines, complement-fixing bodies, and opsonins. Nothing is known of a true antitoxin in this serum. The serum alone will not kill streptococci, but, on the contrary, the bacteria will multiply in it. If, however, after the virulent bacteria have been treated with the serum they are brought in contact with living leukocytes the latter will engulf and destroy them. This is known as phagocytosis, and the serum is said to have opsoninized.
the bacteria. Before the bacteria had been treated with the serum the leukocytes could not take them up. If antistreptococcus serum is injected into a normal animal in suitable amount—2 to 4 c. c. for a guinea-pig—there follows a demonstrable increase of opsonin in the blood, and at this time a dose of living streptococci which would promptly infect and kill a normal control animal is without harmful effect. If the bacteria have been injected into the peritoneal cavity, and after a few hours a little of the peritoneal fluid is drawn off, stained, and examined, a few bacteria will be seen within the abundant leukocytes and none free outside the cells, while in the fluid from the control animal the leukocytes will be few in number and streptococci will appear in abundance free in the fluid outside of the cells where they have multiplied. In guinea-pigs the injection of the serum after the fatal dose of streptococci has been administered has little effect; at the very latest it must not be delayed more than a few hours. In man also a rise of streptococcus opsonin in the blood follows the injection of antistreptococcus serum, and in cases of infection by streptococci this opsonin becomes attached to the bacteria and enables the leukocytes to engulf and destroy them. In cases of severe infection in man the leukocytes lose much of their power of taking up the streptococci, even in the presence of abundant opsonin, and this factor may explain why so little or no apparent benefit follows the use of the serum in some cases of human streptococcus infection. It is, therefore, important that the serum be administered early in the infection before the leukocytes have been so injured as to be relatively inactive.

The first to report the use of the serum of a large animal to combat streptococcus infections was Roger (7). He was soon followed by Marmorek (4). Denys and Leclief (3) a short time afterward reported similar studies carried out independently. These workers produced an antistreptococcus serum from mules and horses through injections of cultures of streptococci which were virulent for animals. The streptococcus used by Marmorek had been rendered highly virulent for rabbits by multiple passage. The sera produced by these first investigators when injected into small animals protected them against infection by the streptococcus used in the immunization. While the protective power of the serum was very distinct, it possessed but little power of rescuing animals previously infected from a fatal termination, and was only able to do so if injected within a few hours after inoculation by the living bacteria. In 1902 Aronson (1) described an antistreptococcus serum which was prepared by injecting horses with a streptococcus rendered very highly virulent for mice by multiple passage. This serum was also able to protect mice against infection with the immunizing culture, but was only able to act in a curative way a few hours after the infection had been produced. The same year Moser (6) put forward what he called a scarlet-fever streptococcus serum. It differed from former sera in its mode of production in
that horses were injected with the streptococci obtained from the heart's blood of fatal cases of scarlet fever without passing them through animals. Since the cultures used were not virulent for animals, it was impossible to determine the protective and curative properties of the serum. Moser proceeded upon the theory that streptococci cultivated directly from man would stimulate the formation in the horse of antibodies which would act against corresponding streptococci in man. This serum was designed to be used in the treatment of scarlet fever.

Following the general methods described by these earlier investigators, commercial producers everywhere have taken up the manufacture of antistreptococcus serum.

The horse has come to be generally employed for the production of antistreptococcus serum in large quantities, and the opsonic content of the serum is larger when living streptococci are finally injected directly into a vein in an amount not large enough to cause an active infection, i.e., in such quantity that they are disposed of without multiplying. The blood of the immunized horse is drawn in a sterile manner from the jugular vein, and after clotting the serum is allowed to separate. The serum is then drawn off, passed through a Berkefeld filter to insure its sterility, and placed in suitable amounts in small glass bottles or tubes, which are hermetically sealed, or tightly closed with rubber stoppers or paraffined corks. It is customary in this country to add 0.3 per cent. tricresol to the serum as a preservative. The serum must be sterile and devoid of toxic effects when injected into animals. The horses used in the production of the serum must be healthy. They are tested with tuberculin and mallein to insure the absence of tuberculosis and glanders, and while under treatment should at intervals receive antitetanic serum to protect them from infection by tetanus. In order that a serum may be active against as many strains of streptococci as possible it is customary to employ for the immunizing injections a mixture of cultures obtained from as wide a variety of human streptococcus infections as possible. Serum resulting from this manner of procedure is known as polyvalent. Different investigators have failed to reach the same results as regards the activity of an antistreptococcus serum against strains of bacteria other than those used in producing the serum. Efforts have sometimes been made to produce a serum by immunizing against strains of streptococci isolated from disease such as rheumatism, etc. In all cases it is necessary to remember that antistreptococcus serum is a specific remedy and acts only in streptococcus infections. Case reports in which no bacteriological diagnosis is included have little value. It is not unlikely that many apparent failures from the injection of antistreptococcus serum are due to faulty bacteriologic diagnoses, or, rather, the absence of any proper bacteriologic examinations.

The rapidity with which the serum enters the blood after injection is
an important factor in determining its therapeutic effects. If it is injected directly into a vein its whole effect occurs almost immediately; if into the muscles it occurs after a few hours, while, if beneath the skin, the full effect is not reached before two days. On this account the serum should be injected directly into the circulation in very urgent cases, and, if this is impossible, into the muscles. For intravenous use a serum without any preservative would be desirable. Spiess (9) and De Bersaques and De Waele (2) have advised the administration of antistreptococcus serum by mouth, and they report favorable results following such practice. Some of the specific antibodies may possibly enter the circulation when the serum is swallowed, as was shown to be the case with diphtheria and tetanus antitoxin by McClintock and King (5).

Experience has shown that large doses of antistreptococcus serum are required to produce curative results, since at best the concentration of opsonin is never very great. Doses of from 30 to 100 c. c. are necessary. The need of a repetition of the injection may be indicated by the clinical symptoms, but it may be pointed out sooner by a fall in the opsonic index after a primary rise, or by a persistent high leukocytosis.

Antistreptococcus serum has been used to a limited extent as a local application to the site of open wounds or surface streptococcus infections. Spiess (9) used it with advantage in the form of a powder (desiccated serum), or as a paste as a direct application to the tonsils, and Sexton (8) has found it very efficient when applied as a moist dressing to infected vaccination ulcers. It has also been used in open wounds infected with streptococci. It appears that a more extensive trial in this local manner would be desirable, as in many cases the serum would thus be brought into direct contact with the infecting bacteria.

Judging from what occurs in experimental animals, antistreptococcus serum should be an efficient prophylactic agent against streptococcus infections in man, and its use before certain operations, such as those about the mouth, after which streptococcus infections are specially liable to occur, seems justifiable. The protection, however, is transient, lasting only about ten days.

There is no official standard for antistreptococcus serum in the United States, and the guarantee of the manufacturer must be relied upon as to its potency. Weaver and Tunnicliff (10) found two active and one inactive out of three American serums purchased in the open market. They also found that three out of seven commercial serums over two years old were still active.

[In order that antistreptococcus serum may be clinically effective large doses are required. The propriety of introducing large amounts of foreign serum into a patient must be considered. In diphtheria, where the use of serum is clearly life-saving, this question does
not arise. In streptococcus infections the antistreptococcus serum is probably of value in some cases, but certainly it cannot be compared in value to antiphtheritic serum. It then becomes a question as to whether the gain from the serum will offset the dangers of the severe reactions which commonly follow large doses of serum, the symptoms of which, when added to those of the septic infection for which the serum is given, may seriously threaten the life of the patient. In selected cases, particularly early in the course of a proved streptococcus infection, the immediate value of serum may be greater than the possible later dangers of serum reaction, but in every instance the various elements in the case must be carefully weighed. In certain cases the antiserum may be recommended, but the routine treatment of streptococcal infections by antistreptococcus serum does not seem to be justified by the collected clinical results.—Editors.]

REFERENCES

7. Roger. Comtes rendus de la société de biologie, 1895.
CHAPTER XXVI

ANTISTAPHYLOCOCCUS SERUM

ERNEST E. IRONS

Thomas (1) reports the results of treatment of 28 cases of infection by microcococcus aureus, including one case of sepsis, with an immune serum obtained from a ram immunized against 18 strains of microcococcus aureus. The serum was rich in opsonins, was given in doses of 1 to 3 c. c. subcutaneously, and appeared to have therapeutic value. In cases of staphylococcal sepsis a potent serum should be of value; heretofore attempts at passive immunization in staphylococcal infection have not been generally successful. As pointed out by Van de Velde (2) in a critical review of staphylococcal sera, the evidence for the specific activity of the sera on the market is very scant, and in most instances consists of the statement of the manufacturers that the serum is recommended for staphylococcal infections.

Deutschmann’s serum, obtained from animals by feeding them with large amounts of yeast, is said to have a favorable effect on infectious processes, though this action is obviously non-specific, and by many the serum is believed to be no more effective than normal serum.

REFERENCES

CHAPTER XXVII

ANTIANTHRAX SERUM

Ernest E. Irons

Antianthrax serum has proved of value in the protection of animals against anthrax, and also in the cure of the disease. The serum has also been used in combination with injections of bacillary or spore vaccine in the prophylactic immunization of animals. The mode of action of the serum is not well understood, but the results obtained with it in animals justify its employment in anthrax infections in man.

Sera having a protective power against artificial anthrax infections are obtained by the immunization of sheep, horses, and cattle. In the treatment of anthrax in animals, the administration of serum is followed by a drop in temperature and decrease in severity of symptoms. A relapse may occur on the second or third day, and calls for further injections of serum. It has been proved that animals affected with anthrax may recover after an injection of potent serum, even after the bacilli are found in the blood (Eichhorn).

Sclavo reports a mortality of 6.09 per cent. in 164 cases of anthrax in man treated with serum, as against a mortality of 24.16 per cent. in cases which received no serum. Other clinicians of experience in this disease in Germany testify to the value of serum (Soberheim). The report of the Bradford Investigation Board in England (1912) favors the use of Sclavo’s serum as a valuable remedy, and refers to four severe cases in which serum was used with recovery.

The serum should be given in doses of 20 to 40 c. c., subcutaneously, or in severe cases intravenously. In desperate cases larger doses have been given with apparent benefit (Soberheim).

The treatment of malignant pustule is primarily surgical, but, where available, serum should be given as well, particularly in view of the evidence of its prophylactic value in animals.

Kolmer recommends the following method of treatment: “If the lesion is relatively small with little or no evidence of toxemia, a blood culture should be made by placing 2 to 5 c. c. of blood in a flask of neutral bouillon. An intravenous or intramuscular injection of 20 to 50 c. c. of
serum is given. The object is to introduce serum before the lesion is
handled, and the blood culture is the best indication for subsequent in-
jections of serum and serves as a guide to prognosis.” The lesion should
be excised with as little handling as possible, and the site dusted with
calomel. In case the edema does not subside and infection is spreading
at the edges of the wound further excision of tissue may be necessary,
and more serum should be injected. The injection of phenol into the
wound has been recommended. If after 24 hours the cultures show
anthrax bacilli, 100 to 200 c. c. of serum are to be injected intravenously.
Daily blood cultures are made, and serum injected until the blood becomes
sterile. Kolmer states that in his experience patients with sterile blood
cultures have invariably recovered, and those with bacilliemia have usually
died.

REFERENCES

CHAPTER XXVIII

GLANDERS

ERNEST E. IRONS

Thus far no efficient specific antiserum has been obtained for the treatment of glanders. The blood of animals suffering from glanders, or immunized by inoculations of the organism, contains specific agglutinins and precipitins, and gives the reaction of complement fixation with antigen derived from B. mallei. All of these serum reactions are of use in the diagnosis of glanders. The mallein reaction is specific and is obtained by a technique similar to that employed in the tuberculin reaction. (See Diagnostic Reactions.)

In the treatment of glanders vaccines prepared from the bacilli may be used in the subacute and chronic cases. Park advises doses of 20- to 200,000,000 at intervals of two to four days. A few cases are on record in which vaccines have been used with apparent benefit. Horses have been successfully immunized by the inoculation of killed cultures. (Silkman, quoted by Park.) Mallein has been used therapeutically, but its value is questioned.

The diagnosis of atypical cases, particularly the chronic forms, presents well-known difficulties, owing to the resemblance of the lesions to ulcerative lesions of syphilis and other granulomata. A case of supposed subacute glanders in a young man who had been associated with horses recently came to the attention of the writer. Cultures from the lesion showed the absence of glanders bacilli and the presence of the organisms of sporotrichosis.

The mallein reaction, the serum reactions of agglutination and complement fixation, the isolation of the organism in cultures will establish the diagnosis. The Strauss reaction in guinea-pigs will assist in the identification of the organism.
CHAPTER XXIX

ROCKY MOUNTAIN SPOTTED FEVER

Ernest E. Irons

In the course of his studies on Rocky Mountain spotted fever, Ricketts used serum from a horse immunized against the disease in the treatment of one case of spotted fever in man. Heinemann and Moore continued this work, and obtained potent immune sera by immunizing horses by subcutaneous and intravenous inoculations of virus obtained from guinea-pigs. This serum in amounts of 1 c.c. protected guinea-pigs against 1,000 fatal doses when given before the first day of high fever in the infected animal. Immune horse serum was concentrated by the methods used for the concentration of diphtheria antitoxin, and after sterilization by filtration was put up in vials of 25 c.c. The serum was used in a number of cases of spotted fever in Montana in daily doses of 5 to 10 c.c. Owing to the fact that but few reports were received from cases in which it was used, the value of the serum in the treatment of the developed disease in man has not been determined. In a personal communication Drs. Moore and Heinemann state that from the reports of some physicians of experience in the diagnosis and treatment of spotted fever it appears that the serum was of value in some of the cases in which it was used. Among the difficulties experienced in estimating the value of the serum in saving life have been the variations of the mortality rate of the disease from year to year and errors in diagnosis from the confusion of measles with spotted fever by inexperienced observers. At present the most promising means of combating the disease appear to be those of prophylaxis, directed against the wood tick, by the bite of which the disease is transmitted to man.
CHAPTER XXX

MALTA FEVER

(Mediterranean Fever—Undulant Fever)

ERNEST E. IRONS

Malta fever is caused by micrococcus melitensis, and presents the symptoms of a septicemia of subacute or chronic course, with periods of continued, remittent, or intermittent fever, enlargement of the spleen, with tendency to relapses, with a frequently associated arthritis, synovitis, parotitis, or orchitis.

Micrococcus melitensis is a very small Gram-negative coccus (0.3μ x 0.4μ), and occurs singly or in pairs. Somewhat elongated forms are sometimes seen, and in old cultures short chains and irregular involution forms may be observed. The organism grows slowly, best at 37° C., and on agar, gelatin, and somewhat better on media containing serum. In media containing carbohydrates, including dextrose or lactose, the micrococcus melitensis does not form gas; after several days the media become slightly alkaline. Gentry and Ferenbaugh (2) isolated the organism from the blood by inoculating small quantities of blood (.3 to 2 c. c.) into flasks containing 10 to 50 c. c. of broth, and into litmus milk. After incubation for three days at 37° C. subcultures were made on glucose-nutrose-litmus-agar.

The micrococcus can be readily isolated from the blood during the accessions of fever. Infection occurs through the ingestion of milk of infected goats, and possibly through wounds in the hands of those caring for goats, or through the inhalation of dust from the pens in which the animals are kept. (Gentry.) The micrococcus has been isolated from the stomachs of three species of mosquito, culex, stegomyia, and acartomyia found at Malta, and it is possible that infected mosquitoes in rare instances may be the source of infection in man.

Malta fever is met with in a number of Mediterranean districts, in Africa, Asia, and South America. It occurs also in the Philippines, and in recent years has been shown to be endemic in the southern portion of Texas in districts in which goats are extensively raised.

Gentry and Ferenbaugh have studied the occurrence and distribution
of the disease in man, and in goats in Texas, where it is often referred to as "goat-fever." Unrecognized cases usually are regarded as atypical forms of typhoid fever. Yount and Looney (4) have reported five cases from Arizona. The cases in Texas and Arizona have occurred for the most part in goat herds or in persons associated with the animals.

IMMUNITY

It is probable that one attack of Malta fever in most instances confers a lasting immunity; the relapses, with two or more febrile periods, usually less severe than the initial attack, suggest that immunity is only partially developed for weeks after the onset. Specific agglutinins appear early, sometimes the first to fifth day of symptoms; and in dilutions 1-100 to 1-1000 and higher. The agglutination titre may rise more tardily, but eventually often reaches high dilutions. The appearance of specific agglutinins in man, and in animals used for experimenting, may antedate by some weeks the establishment of immunity and permanent recovery from the disease. This is well shown in the following chart by Eyre (1):

![Graph showing temperature and titre of serum with respect to agglutinins.]

**(Fig. 1.—Curves of Temperature and Titre of Serum with Respect to Agglutinins.** C. John, age 24. Sailor. Recovery. Heavy line—Temperature. Fine line—Titre of serum with respect to agglutinins. (From Eyre. Mittelmeerfieber, Hanb. d. Microörg., Kolle und Wassermann, 1913, iv. 436.)

Specific complement-fixing substances are demonstrable in the blood of infected persons and animals, and in the blood of animals immunized against the disease. (Mohler and Eichhorn, 3.)

DIAGNOSIS

In regions where the disease is endemic the diagnosis may present no difficulties. The use of milk of goats, or association with the animals and the prolonged course, with enlarged spleen and frequent localizations of the infection in joints, are suggestive. Atypical and sporadic cases will present greater difficulties and are undoubtedly often missed. A positive diagnosis is made on the finding of the specific organism in the blood, often in the urine, and occasionally in acute cases in the feces, and on the serum reactions. Blood cultures are often positive from the second day of symptoms—68 per cent. in 103 cases (Shaw); 82 per cent. in 45 cases.
REFERENCES

(Gilmour). The micrococcus is frequently found in the urine, and has been isolated from effusions in affected joints.

The agglutination test is carried out in typhoid fever, using suspensions of the micrococcus melitensis. Dilutions of the serum of 1-40 and higher are used. Normal sera from man and from goats are occasionally met with which agglutinate the micrococcus in dilutions of 1-10 or 1-20, and therefore higher dilutions, 1-40, 1-100, should be used in making the test in a suspected case.

Complement-fixation, using an antigen prepared from the micrococcus, may be employed in diagnosis. (Mohler and Eichhorn.)

PROPHYLACTIC INOCULATION

Eyre immunized 51 men with killed micrococci in doses of four million, repeated two or three times. In the following four months none of these developed the fever, while of 58 other control persons three became ill with Malta fever. Later, however, two of the immunized persons became ill. This result indicated the production of an immunity of short duration. Larger doses will probably confer a more lasting immunity.

TREATMENT

Serum.—Serum from an immunized horse was used in the treatment of experimental infection in monkeys, but the serum did not appear to influence the course of the disease in the inoculated animals as compared with infected control animals which did not receive serum. One case of Malta fever in man was given serum without any marked result. (Eyre.)

Active Immunisation by Killed Cultures.—Reid reported good results in the treatment of Malta fever by vaccine. Basset-Smith believed that inoculations were of value in the chronic cases, but perhaps harmful in the acute. Eyre has used small doses (5- to 10,000,000), not only in chronic, but in acute cases developing on their return to England, and believes that the inoculations decrease the severity of the febrile attacks and shorten the course of the disease. Other workers have recommended larger doses up to 50,000,000 killed cocci.

REFERENCES

CHAPTER XXXI

HODGKIN'S DISEASE

Ernest E. Leons

For years students of Hodgkin's disease have been divided in their opinions as to its nature, some maintaining that it is a form of malignant tumor, others that it is infectious in origin, and that the granulomatous changes in glands are due to microorganisms or their toxins. Sternberg maintained that it is due to toxins of the tubercle bacillus.

Fraenkel and Much (6) found Gram-positive, non-acid-fast bacillary and granular bodies in the residue of glands treated by antiformin in a number of cases.

Kusunoki (7) examined the tissues from 16 cases of typical lymphogranulomatosis by the antiformin method, and by stained sections, and found non-acid-fast Gram-positive rods in all. He searched carefully for acid-fast organisms, but was unable to find them except in one lymph node, which showed the histology of tuberculosis and probably constituted a small focus of tuberculosis occurring in the subject of a lymphogranulomatosis. He was unable to find the typical Gram-positive rods in normal lymph nodes, spleen, nor in the nodes of lymphosarcoma. Kusunoki also reviewed the studies of others on this subject.

In 1913 Negri and Mieremet (9) described a pleomorphic organism resembling somewhat the diphtheria bacillus which they isolated from two cases of Hodgkin's disease and suggested the name corynebacterium granulomatis maligni.

Bunting and Yates also isolated a diphtheroid organism from the granulomatous masses of Hodgkin's disease and studied the lesions in monkeys produced by the inoculation of cultures of the organism.

Rosenow studied 35 cases of Hodgkin's disease and isolated the diphtheroid bacillus in 34 cases. Other organisms were also obtained from these cases as follows: staphylococci in 16 cases; streptococci in 6 cases; a bacillus resembling B. Welchii in 8 cases; Gram-negative bacilli in 4 cases. In two cases blood cultures yielded the diphtheroid organism, once in pure culture and once in association with a streptococcus. In four cases a probable infection atrium was found; three times in alveolar abscesses and once in a cavity in the tonsil.
HODGKIN’S DISEASE

Coley (4) maintains that the clinical features of Hodgkin’s disease resemble so closely those of sarcoma that it is often impossible to differentiate the two conditions. The histology of the glands in the two conditions may be so similar that pathologists are divided as to whether a given specimen is Hodgkin’s disease or sarcoma. Coley concludes: “The fact that Hodgkin’s disease and leukemia have certain features pointing to an infectious origin should not exclude them from being classed as malignant tumors; but on the contrary this fact furnishes additional evidence in favor of the infectious origin of sarcoma.”

On the basis of this conception of the relation of Hodgkin’s disease to sarcoma Coley has employed the toxins of the streptococcus and B. prodigiosus (Coley’s toxins) in the treatment of Hodgkin’s disease, and reports two cases in which the masses disappeared under the treatment (5). The inoculations of the toxins were given subcutaneously at intervals of two or three days, or longer, depending on the reaction, beginning with one-fourth minim at a dose and gradually increasing to twelve minims. One case received inoculations for five months, at which time the symptoms had all disappeared; he appeared to be in good health, and remained so up to the time the report was made three years later.

It is well recognized that the dividing lines between the various lymphomas, lymphosarcomas, and leukemias are by no means well defined, and in the latter, particularly in the acute leukemias, an infectious etiology seems possible.

Nevertheless, the finding of the same bacilli in typical Hodgkin’s disease and in cases clinically and histologically lymphosarcoma, and the occurrence of other organisms such as staphylococci with the bacilli in Hodgkin’s disease, suggest that all these bacteria may be merely secondary invaders which have been filtered out from the blood. The more carefully the bacteriology of the blood is studied the more frequently do we find bacteria present as temporary or intermittent invaders, and it is not surprising that studies of lymph-nodes should show organisms of various sorts, the presence of which has been hitherto unsuspected. The bacteriologic findings in Hodgkin’s disease have stimulated the search for diphtheroids in other tissues, normal and pathologic, unassociated with Hodgkin’s disease, with the result that these bacteria have been found to be widely distributed in the body. While it is possible that the febrile periods noted so frequently in Hodgkin’s disease may be due to the activity of these secondary invaders, the evidence at hand seems to indicate that we must look elsewhere for the primary etiologic agent of Hodgkin’s disease.

J. J. Moore (8) was unable to obtain complement-fixation with the serum of persons suffering from Hodgkin’s disease, using antigens prepared from the diphtheroid bacilli, although he did obtain such reactions in horses, monkeys and rabbits, following immunization with the bacilli.
After an extensive study of fifty cases of Hodgkin's disease, in which the patients were treated with vaccines either alone or in combination with Roentgen rays and arsenic, Billings was able to observe no permanent benefit from vaccines prepared from the organisms isolated. In those cases in which improvement followed, relapse occurred later, so that the temporary initial improvement seemed attributable to spontaneous regressions, or to the effects of the Roentgen rays and arsenic, rather than to vaccines.

REFERENCES

CHAPTER XXXII

THE SPECIFIC TREATMENT OF HAY FEVER (POLLEN DISEASE)

KARL K. KOESSLER

Any endeavor to obtain the specific treatment for a disease rests fundamentally upon the clear understanding of the etiological agent involved and upon the intricate mechanism of its action. The etiological agent of hay fever, the pollen of certain plants, holds a singular position as "infective" agent—a cell, destined from nature to play the most important rôle of the plant organism by multiplying the species, yet a cell which differs from all cellular micro-organisms that cause infectious diseases by lacking completely the faculty of multiplying within the invaded host. A formed poison then? A poison proving completely indifferent to the greater part of mankind, though one of the most intense poisons to some.

This particular place which we must concede to the etiological factor of pollen disease, calls for a preliminary review of the problem of etiology, pathogenicity, individual disposition, local tissue-immunity, and general immunity, before the specific treatment can be discussed.

HISTORICAL REVIEW 1

The recognition of hay fever as a special clinical entity dates from March 16, 1819, on which day John Bostock (12), an English physician, reported to the Royal Medical and Chirurgical Society of London, his own history of yearly suffering as a "case of a periodical affection of the eyes and chest." Since this record contains almost all the clinical symptoms which we consider characteristic of the affection to-day—a fact which has been recognized by the German school in giving to hay fever the cognomen "Bostock's catarrh"—it will be given place here instead of any other symptomatology of the disease.

The following case, it is presumed, will not be altogether uninteresting to the Society, as affording an example of an unusual train of symptoms, and it may perhaps be considered the more worthy of their attention from its having occurred in the person of the narrator:

1 No complete history of hay fever is intended here but only those phases shall be given which pertain to its recognition as pollen disease.
J. B., set. 46, is of a spare and rather delicate habit, but capable of considerable exertion, and has no hereditary or constitutional affection, except various stomach complaints, probably connected with, or depending upon, a tendency to gout. About the beginning or middle of June in every year the following symptoms make their appearance, with a greater or less degree of violence. A sensation of heat and fulness is experienced in the eyes, first along the edges of the lids, and especially in the inner angles, but after some time over the whole of the ball. At the commencement the external appearance of the eye is little affected, except that there is a slight degree of redness and a discharge of tears. This state gradually increases, until the sensation becomes converted into what may be characterized as a combination of the most acute itching and smarting, accompanied with a feeling of small points striking upon or darting into the ball, at the same time that the eyes become extremely inflamed and discharge very copiously a thick mucous fluid. This state of the eyes comes on in paroxysms, at uncertain intervals, from about the second week in June to the middle of July. The eyes are seldom quite well for the whole of this period, but the violent paroxysms never occur more than two or three times a day, lasting an hour or two each time; but with respect to their frequency and duration there is the greatest uncertainty. Generally, but not always, their invasion may be distinctly traced to some exciting cause, of which the most certain is a close moist heat, also a bright glare of light, dust or other substances touching the eyes, and any circumstance which increases the temperature. After the violent inflammation and discharge have continued for some time, the pain and redness gradually go off, but a degree of stiffness generally remains during the day.

After this state of the eyes has subsisted for a week or ten days, a general fulness is experienced in the head, and particularly about the fore part; to this succeeds irritation of the nose, producing sneezing, which occurs in fits of extreme violence coming on at uncertain intervals. To the sneezings are added a farther sensation of tightness of the chest, and a difficulty of breathing, with a general sensation of the fauces and trachea. There is no absolute pain in any part of the chest, but a feeling of want of room to receive the air necessary for respiration, a huskiness of the voice, and an incapacity of speaking aloud for any time without inconvenience. To these local symptoms are at length added a degree of general indisposition, a great degree of languor, an incapacity for muscular exertion, loss of appetite, emaciation, restless nights, often attended with profuse perspirations, the extremities, however, being generally cold. The pulse is permanently quickened, from 80, the average standard, to about 100, and upon any considerable exertion it rises to 120 or more.

This is an account of the complaint in its worse state, which, however, it does not assume in every season, and indeed its violence is generally less than is here described. The affection of the eye is recollected to have occurred when the patient was 8 years old, and there has been more or less of it every year since; the sneezing came on early at the same period, but the first attack of the chest was at the age of sixteen or seventeen. Generally speaking, the complaints have increased for the last 20 years, although not progressively. All the acute symptoms disappear about the end of July, but a considerable degree of weakness and languor is left, which remains a month or six weeks longer. It has happened that the most severe summer complaints have been experienced after the patient had enjoyed the best health during the preceding spring. On the contrary, it has been thought that after a severe summer attack, the patient has more completely and more rapidly regained his usual state of health and strength in the autumn.

The remedies employed have been various, and they have been persevered in
with an unusual degree of steadiness. Topical bleeding, purging, blisters, spare diet, bark and various other tonics, steel, opium, alterative courses of mercury, cold bathing, digitalis, and a number of topical applications to the eyes, have been very fully tried, and it is doubtful whether any distinct or permanent benefit has been derived from any of them. The complaint once seemed to be decidedly stopped by a journey, but in other instances it has existed while the patient was traveling. By using every means of obtaining fresh air without much exertion, and by carefully avoiding a moist and close atmosphere, the symptoms may in some measure be kept off, but they have frequently appeared under circumstances that seemed the least likely to have produced them.

It may form an important addition to the narrative to state that during the last summer the patient was so situated as to be able to avoid almost every degree of bodily exertion; he remained nearly confined to the house for about six weeks, and the result was that, notwithstanding the unusual warmth of the season, he experienced much less of the affection than he had done for several years before.

Bostock (13) published later, in 1828, a fuller account of the disease, which contains the additional record of several new cases. In this publication he first uses the name "catarrhus aestivus," summer catarrh, and mentions that the name hay fever, which he had not used in his first communication, has now been commonly used for the affection for several years. It is important to note that Bostock thought that the affection was due to heat and sun rays, and not to the effluvium of fresh hay, an opinion which prevailed at large in the popular mind of those days.

That there exists a certain form of catarrh of the mucous membranes of annual and seasonal periodicity, which is correlated in some respect with the flowering period of plants, is an idea which finds expression in the works of medical writers of the sixteenth, seventeenth, and eighteenth centuries—hundreds of years before Bostock's publication.

Leonhardus Botallus (14) of Pavia, seems to be the first medical author who mentions a condition which suggests hay fever. He tells, 1565, of patients who had an intense aversion to roses, since their odor caused them headache, itching of the nose, and sneezing.

Joannes Baptista von Helmont (31), 1577–1644, tells of a cleric who suffered every year from asthma, but only during the summer months, being completely free from it for the rest of the year.

Konrad Victor Schneider (45), professor in Wittenberg, was the first to demonstrate that nasal catarrh depends on the active exudation of the nasal mucosa, and not, as Hippocrates and Galenus thought, on a secretion of the brain—an opinion which still finds its expression in the French rhume de cerveau for common cold. He mentions in his treatise on the various forms of catarrh, 1662, the rose catarrh: Rosa olfactata movet catarrhos et sternulationes.

Joannes Nicolaus Binningerus (2) in 1673, reports the case of a lady of apparently substantial frame of body, ampli corporis et carnosi, who suffered every year at the time when the roses flowered, from coryza for several weeks.
Samuel Ledelius (35) in 1684, reports the case of a merchant of melancholic temper, who had itchings of the eyes followed by inflammation and severe headaches every year at the time when the roses bloomed. He observed that he could avoid these disagreeable symptoms if he remained at home at this season of the year.

Jacobus Constant de Rebque (18) in 1691, recounts that he himself suffered for thirteen years during the time of flowering of roses from a coryza which ended only with the deflorescence of these flowers. He suspected that the ailment was induced by some product of the roses and termed it coryza a rosarum odore.

A similar case is reported by Riedlin (44) in 1695.

William Heberden (29), 1710-1801, the earliest author referred to by Bostock, observed the annual periodical occurrence of catarrh "in four or five persons in the months of April, May, June or July, and last a month with great violence."

All these observations suggest that a seasonal catarrh at the time of the blooming of roses (rose cold) was known to some observers long before Bostock, yet all these reports refer to the condition as a mere medical curiosity and do not plead for it as a clinical entity. To have established hay fever as a true clinical entity is the lasting merit of John Bostock. About the time of his second publication there appeared some communications on hay fever which are of great interest regarding the question of the etiology of the disease.

John MacCulloch (37) in 1828, published in London "An Essay on the Remittent and Intermittent Diseases," in which he states that the disease is produced by hothouses or greenhouses, and in the public estimation, particularly by hay fields. In the following year W. Gordon (28) published an article in which he incriminated the aroma emitted by the fresh flowering grasses, especially by the blossoms of the sweet-scented vernal grass, antoxanthum odoratum, as the causative factor of hay asthma which he says should be termed preferably grass asthma.

The most important communication regarding the etiology of hay fever in the first half of the nineteenth century comes from John Elliotson, (25), a very well known physician and medical writer of his time. He was the first to pronounce (1830) that the disease did not depend upon hay and therefore should not be called hay fever; but that it depended solely on the fresh flower of grasses and probably upon the pollen.

Outside Great Britain, the first communications came from France, where Cazenave (15) of Bordeaux, in 1837, unaware of the publication of the English authors, gave a very correct description of the characteristic symptoms of the disease, which he, however, ascribed to the effect of light.

The literature on hay fever in the next twenty years, though relatively expansive, contains very little which advanced the knowledge of its etiology, pathogenesis or nosography. But the subject was discussed with interest
HISTORICAL REVIEW

from then on, and this helped materially to establish finally the clinical entity of the disease. However, the history of hay fever, like the history of any human achievement, has never lacked those vague spirits who derange and confuse the picture which clear insight and painstaking labor have unridded from the bewildering multitude of natural phenomena, or who concertedly deny the very existence of anything which their own eyes have never had occasion to see, or are incapable of seeing.

In the United States hay fever was known much earlier than is usually assumed and reported, a very obvious fact when we consider the intimate intellectual exchange between England and America. Thus in Robley Dunglison’s (24) “Practice of Medicine,” which was published in Philadelphia in 1842, we find a good account of hay fever (summer bronchitis), the author adding one personal observation to the subject; otherwise his description is based on the works of the first English contributors. In 1844 there appeared an American edition of John Elliotson’s (26) “Principles and Practice of Medicine,” a very excellent work, in which eight pages are given to the discussion of hay asthma, including the report of a case in a farmer’s wife. In this observation, he refutes the statement of his contemporaries that hay fever is a disease which is confined only to the upper ranks of society. He says: “It would be very odd if it were confined to the higher orders only. It is a thing exceedingly improbable. The fact is, the lower orders consider the hay fever as merely a common cold and they do not apply for medical advice unless they are seriously ill. They do not think of applying to public charity because they are seized with violent sneezing; or, if they do, it is a solitary case and is treated as asthma, the nature and causes of the disease not being known.”

Dunglison, as well as Elliotson, speaks only of the early type of hay fever, the rose or June cold, which commences the last week in May or the first week in June and continues until about the second week in July—the catarrhus aestivus as described by Bostock. We know to-day that this form of hay fever is far less frequent on our continent, and also much less dreaded than the autumnal catarrh, the common American form of hay fever, which commences in the second week in August and lasts until the first week of October. That there exists a form of hay fever in the United States differing in the time of onset and in other important features, from the hitherto described English hay fever, was soon recognized by American observers. Thus G. B. Wood (59), in 1849, describes the case of a periodical bronchitis which returned in patients every August “without any assignable cause.” In 1852, Swell (49) gives the first clear differential description of the two forms of the disorder in the United States, a merit which is usually wrongly attributed to Morril Wyman (60), who published an excellent monograph on autumnal catarrh in 1872, by far the best clinical analysis of the disease in our own medical literature. Wyman, though not an absolute adherent of the pollen theory,
was one of the first to recognize in the pollen of ragweed (Roman wormwood, ambrosia artemisiifolia) one of the causative agents of the disease.

Another American monograph appeared four years later written by G. M. Beard (1). He comes to the conclusion that hay fever has to be classed among the functional diseases of the nervous system. Regarding the actual causal agent of the disease, he states that "the number of special exciting causes of hay fever is very large." This attitude of Beard is explained by the then generally reigning opinion regarding the etiology of the disease which comprised all possible factors which could be thought of, but it is nevertheless remarkable since Beard was well acquainted with the experimental researches of Charles Blackley, which fall in the years between 1856 and 1877, through which he established definitely that the pollen is the etiological agent of hay fever. Charles H. Blackley (5-11), a physician in Manchester, England, on the basis of exact analytical observation followed by very numerous and exceedingly cumbersome experiments, advocates the view that hay fever is exclusively caused by the pollen of graminacea. Being subject to the disease, he tested on himself the pollen of about one hundred different species of grasses and flowers, in the fresh as well as in the dried condition, in different ways. He applied it to the mucous membranes of the nasal cavities, he inhaled it, he made a pollen extract and instilled it into the conjunctivæ, applied it to the lips, tongue, and pharynx, and finally inoculated "the upper and lower limbs with fresh moistened pollen." Even when a minute quantity of pollen, as 1/200 gr., was applied to the mucous membrane of the nose, hay fever symptoms appeared in every instance. The pollen of rye caused more violent symptoms than that of grasses. The pollen of wheat and oats had about the same activity as that of grasses, while the pollen of barley had less power. The inhalation of the pollen of certain plants and grasses produced asthmatic attacks and constitutional symptoms. One drop of an extract of the pollen of gladiolus instilled into the conjunctival sack was followed almost instantaneously by burning, itching, and the feeling of the presence of sand in the eyes; in six minutes edema of the conjunctiva and lids followed, but in 32 hours every trace of the trouble had disappeared. Applied to the pharynx, itching and congestion was produced within a half hour. Blackley performed further the first skin test with pollen. On rubbing the pollen into the scarified skin of the forearm and over the tibia, erythema with intense itching and swelling resulted, which lasted for four days. These effects, though common to all different kinds of pollen, varied considerably in intensity. The poisonous properties of plants stood in no relation to the effectiveness of their pollen in producing hay fever symptoms. Blackley observed further that a high temperature favored the growth of pollen, while a low temperature was unfavorable. From these experiments Blackley concluded that: (1) the pollen produces catarrhal and asthmatic symptoms in himself; (2) that the effect though
varying in degree, is common to the pollen of all plants experimented with, but the odor of graminaceae possesses it in a markedly higher degree than the others. He raises the question if the granular matter of the pollen might not find its way through the mucous membranes of the respiratory tract and thus give rise to the constitutional disturbances that are noticeable in some cases.

Having established these facts Blackley proceeded to investigate the relation between the quantity of pollen in the atmosphere and the intensity of symptoms. He exposed to the wind glass plates covered with a sticky mixture, and determined how many pollen grains were deposited in 24 hours. Experimenting between the months of April and August (1866), he found that between May 30 and August 1 the quantity of pollen increased gradually up to the last week in June, and then decreased. In his own case the symptoms of the disease rose and fell in exact correspondence to the increase or decrease of the amount of pollen in the atmosphere. He observed further that after a rainfall the quantity of pollen was considerably lessened. He observed that about 95 per cent. of all the pollen found on his glass slips belonged to the order of graminaceae. Inside his house, if the windows were closed, he found very little pollen. He established further that the very small amount of pollen in the atmosphere, observed prior to June 8, did not produce hay fever symptoms. He repeated all these experiments in 1867 and 1869, and in the country as well as in the city, and observed that in the city there was on the average 1/10 as much pollen as in the country.

Blackley, who published his "Experimental Researches on the Cause and Nature of Hay Fever in 1873," had occasion later to repeat and confirm his investigations on a large number of hay fever patients. As his numerous publications up to 1898 show, he held to his views that pollen is the only etiological factor of hay fever, undisturbed by the tendencies of his time to see in hay fever a bacterial disease.

Under the influence of the etiological researches of Pasteur and Koch, hay fever began to be considered an infectious disease. Herman Helmholtz, who suffered from the disease in a slight degree, described vibrio-like organisms which he considered the causative agent of the disease. Binz and his pupil Patton confirmed and accepted this view. Heyman and Matzuschita thought that the pollen acted as a carrier of hay fever bacteria, especially streptococci, but later abandoned this idea. It is useless to devote more space to the discussion of the bacterial theory of hay fever. Not one of Koch's postulates has been fulfilled in any of the experiments described.

Yet Blackley's pollen theory was by no means at once generally accepted in the United States or Europe. In Germany George Sticker (47), professor in Bonn, one of the best students of the whole problem, published in 1896 his remarkable monograph on hay fever, which must be
considered a standard work on the subject. He, too, accepting the observations of Helmholtz (30) and Binz (3), considered hay fever an infectious disease and expressed his conviction that the true parasite would soon be found. Heyman and Matzschita (32), Thost (50-51) and Weil (57), described different micro-organisms. But all these opponents which Blackley had at the beginning of the twentieth century were finally convinced and converted to his views by the investigations of Dunbar (19), Director of the State Hygienic Institute in Hamburg. Dunbar, himself a sufferer of hay fever, published in 1903 his first paper, which soon was followed by many others, from his own pen and from those of his pupils, Weichhardt (58), Praussnitz (40) and Kamman. He accepted the pollen theory of Blackley, repeated his main experiments and elaborated them by new ones. He isolated "what he thinks is a true pollen toxin" with which he was able to produce an attack of hay fever at any time of the year in persons susceptible to the disease, and finally instigated by the work of von Behring, he prepared his "specific serum" against hay fever. Dunbar's work on hay fever has not only established beyond doubt the rôle of the pollen as the etiological agent, but by applying the modern methods of immunology to the problem, he has given to it a real scientific fundament on which any future work on hay fever has to be based.

DEFINITION

Hay fever is an exudative catarrh of the conjunctival, nasal, and tracheo-bronchial mucous membranes of seasonal periodicity, produced in hypersensitive individuals by the sensitizing and anaphylatoxic action of the pollen of certain plants.

ETIOLOGY

If it can be considered as absolutely proven that the pollen of certain plants is the causative agent of hay fever, the problem is immediately raised: which kind of pollen is the most dangerous for hay fever patients? Which is related to the catarrhus aestivus (rose cold), and which to the autumnal catarrh? The best studies on this subject are necessarily incomplete. It is not easy to find many patients who are willing to have these repeated tests, causing discomfort and irritation, made, for they are subjects whose nervous system is often in too labile an equilibrium to yield to altruistic appeals. Our true knowledge is largely due to the self-sacrificing attitude and interest of physicians, who, themselves subject to the ailment, have not hesitated to test their own theories on themselves. Thus Blackley, Wyman, Marsh, Dunbar, and Praussnitz performed many hundred tests on their eyes and noses and taught us in this way facts regarding the poisonous properties of pollen which otherwise
we should not have known at all; or indeed, only from the subjective statements of patients, which need the objective verification of the investigator to become of scientific value. The pollens of many plants were thus examined and those interested in this question will find the complete list in the works of these authors. Their experiments, however, have naturally been made mostly on patients suffering from the European type of hay fever, and have therefore only conditional value for the United States.

Of the grasses and flowers which have primarily to be considered in the American pollen disease, the following list gives the most important. It is compiled partly from experiments of other investigators, and partly from my own experiments carried out during the last four years.

Patients suffering from June cold (rose cold, summer catarrh, *cata-rhus aestivus*) were susceptible to the pollen of the following grasses:

<table>
<thead>
<tr>
<th>Graminaceae</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Alopecurus pratensis</td>
<td>Meadow Foxtail</td>
<td>May to July</td>
</tr>
<tr>
<td>Anthoxanthum odoratum</td>
<td>Sweet vernal grass</td>
<td>April to July</td>
</tr>
<tr>
<td>Avena sativa</td>
<td>Common oat</td>
<td>June to July</td>
</tr>
<tr>
<td>Cynodon dactylon crinitus</td>
<td>Crested Dog's Tail Grass</td>
<td>June to August</td>
</tr>
<tr>
<td>Festuca octoflora</td>
<td>Slender fescue</td>
<td>May to August</td>
</tr>
<tr>
<td>Festuca rubra</td>
<td>Red fescue</td>
<td>June to August</td>
</tr>
<tr>
<td>Festuca elatior</td>
<td>Meadow fescue</td>
<td>June to August</td>
</tr>
<tr>
<td>Hordeum sativum</td>
<td>Common barley</td>
<td>June to August</td>
</tr>
<tr>
<td>Lolium perenne</td>
<td>Ray grass</td>
<td>June to July</td>
</tr>
<tr>
<td>Phleum pratense</td>
<td>Timothy, Cat's Tail</td>
<td>June to August</td>
</tr>
<tr>
<td>Poa annua</td>
<td>Low spear grass</td>
<td>April to October</td>
</tr>
<tr>
<td>Poa pratensis</td>
<td>Kentucky blue grass, or</td>
<td></td>
</tr>
<tr>
<td></td>
<td>June grass</td>
<td></td>
</tr>
<tr>
<td>Poa tritice</td>
<td>False red top</td>
<td>May to August</td>
</tr>
<tr>
<td>Secale cereale</td>
<td>Rye</td>
<td>June to August</td>
</tr>
<tr>
<td>Triticeum sativum</td>
<td>Wheat</td>
<td>June to July</td>
</tr>
</tbody>
</table>

Patients suffering from the autumnal catarrh were susceptible to the pollen of the following dicotyledones:

<table>
<thead>
<tr>
<th>Dicotyledones</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambrosia artemisae folia</td>
<td>Ragweed</td>
<td>July to September</td>
</tr>
<tr>
<td>Ambrosia bidentata</td>
<td>Great ragweed</td>
<td>August to September</td>
</tr>
<tr>
<td>Ambrosia trifida</td>
<td>Starwort</td>
<td>July to September</td>
</tr>
<tr>
<td>Aster</td>
<td></td>
<td>August to October</td>
</tr>
<tr>
<td>Chrysanthemum Leucanthemum</td>
<td>Oxeye daisy</td>
<td>June to September</td>
</tr>
</tbody>
</table>
### SPECIFIC TREATMENT OF HAY FEVER

**DICOTYLEDONES—Continued**

<table>
<thead>
<tr>
<th>Chrysanthemum Indicum</th>
<th>Common thistle</th>
<th>August to October</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cirsium lanceolatum</td>
<td>Canada thistle</td>
<td>July to October</td>
</tr>
<tr>
<td>Cirsium arvense</td>
<td>Blackeyed Susan</td>
<td>July to September</td>
</tr>
<tr>
<td>Rudbeckia hirta</td>
<td>Goldenrod</td>
<td>June to August</td>
</tr>
<tr>
<td>Solidago caesia</td>
<td>Goldenrod</td>
<td>August to September</td>
</tr>
<tr>
<td>Solidago Canadensis</td>
<td>Goldenrod</td>
<td>August to September</td>
</tr>
<tr>
<td>Solidago Nemoralis</td>
<td>Graminaceae</td>
<td>August to September</td>
</tr>
<tr>
<td>Zea Mais</td>
<td>Indian Corn</td>
<td>July to August</td>
</tr>
</tbody>
</table>

Of many other kinds of pollen examined, none was found to be harmful. Yet it would be a grave error if anyone should consider that the above, or any other list, is complete and embraces all the pollens which are pathogenetic for hay fever patients. The hypersusceptibility of these against the pollen protein is certainly individually different in degree, and the pollens of certain flowers, which have been found innocuous to most patients, might in some instances prove harmful to others. Yet the investigation of this problem has led to a number of facts which must be considered of greatest importance for the question of pollen specificity and specific treatment.

If we study the botanical family and class to which the species named above belong, we find that almost all the pollens which have been found to produce the symptoms of June cold belong to the family of graminaceae (monocotyledones), while the flowers whose pollen is pathological for patients who suffer from autumnal catarrh belong almost entirely to the large family of compositae (dicotyledones). Now it is a fact long known in the United States that patients suffering from June cold are only rarely and exceptionally subject to autumnal catarrh and vice versa. Patients who react to the conjunctival instillation of pollen extract of timothy, do not react to the pollen extract of ragweed. The affection known as June cold is, therefore, etiologically, completely different from the autumnal catarrh, and the differences between the two varieties which have been established on the ground of clinical and experimental evidence, may be expressed in the following sentences:

1. The spring variety of hay fever, June cold, rose cold, summer catarrh, Bostock’s catarrh, is due to the pollen of the common field grasses named above, all of which belong to the family of graminaceae.

2. The pollen of these grasses shows structural and morphological similarities. It has a smooth surface and is round or oval in form, presenting an outer membrane, the exine, and an inner one, the intine. The exine shows a reticulate structure, and is easily stained with anilin dyes;
the intine is composed mainly of cellulose, does not stain with anilin dyes, and includes the pollen content or fovilla.

(3) The pollen of the graminaceae contains a large number of rod-like masses which by their dark blue coloration on the addition of lugol solution prove to consist of starch.

(4) These rods of starchy composition, characteristic of most graminaceous pollen, are not the carrier of the poisonous principle, as first held by Dunbar; this is inherent in the albuminous part of the pollen protein.

(5) The chemical composition of the poisonous group and its combination with the rest of the pollen proteins is extremely similar in all graminaceous pollen.

(6) An individual, subject to June cold, will react to the protein extract of any of the graminaceae pollens to about the same degree (conjunctival, nasal and intracutaneous tests), but will not react to the pollen extract of the composite.

(7) Animal experiments which the author made on guinea-pigs confirm this observation in man. Guinea-pigs sensitized to the pollen extract of anthoxanthum react almost as strongly to the reinjection of the pollen of phelum as to the reinjection of the pollen of anthoxanthum, but do not react at all to the reinjection of the protein extract of pollen of ambrosia.

(8) The sensitizing action of the pollen is therefore specific, but specific only within the limits of a group reaction.

(9) The common type of hay fever in the United States—autumnal catarrh—occurs in August and lasts until October.

(10) Autumnal catarrh is due to the pollen of flowers of the composite family, especially to those named above.

(11) The pollen of these flowers is considerably smaller in size than the graminaceae pollen; it has a rough surface with spike-like processes and does not contain any of the rod-like bodies, consisting of starch, present in the graminaceae pollen.

(12) An individual subject to autumnal catarrh reacts at any time of the year to the protein extract of any of the composite named, but does not react to the pollen of grasses.

(13) Experiments on animals confirm this observation. Guinea-pigs sensitized to ragweed pollen react strongly on reinjection after three weeks to goldenrod pollen (though not as strongly as to ragweed pollen), but do not react to reinjection with timothy pollen.

(14) The instillation or inhalation of graminaceae or composite pollen in healthy persons not subject to hay fever, never produces hay fever symptoms.

(15) The incriminated pollen at the time of hay fever is present in the air in sufficient amount to be able to produce hay fever.
(16) The severity of attacks is directly proportional to the quantity of pollen in the air.

(17) Since the degree of poisonous activity of the pollen is in direct relation to the quantity of pollen in the air, ragweed must be considered the chief cause of autumnal catarrh. (Author's experiments in Chicago and vicinity.)

PATHOGENICITY, INDIVIDUAL SUSCEPTIBILITY AND IMMUNITY

In the month of August all inhabitants of a community inhale onto their conjunctival and nasal mucous about the same amount of pollen. The great majority of them remain completely unaffected, while a small proportion become ill with hay fever. The pollen protein is then a substance harmless and indifferent to the greater part of mankind, but of poisonous character to some persons; these possess a certain individual susceptibility or disposition to hay fever, while the great majority of persons are immune to the disease.

The mechanism of bacterial infection and the laws governing immunity reactions show phenomena of apparent similarity. If the water supply of a community is polluted by a large amount of typhoid bacilli only a small proportion of the inhabitants become sick with typhoid fever. This fact is interpreted primarily on the ground that the living micro-organism does not find favorable conditions for its growth and multiplication in most individuals. We thus relate the individual disposition for the disease to the varying pathogenicity of the invading micro-organism. The pathogenicity and virulence of a bacterium is certainly dependent on its ability to grow and multiply within the animal body, but this definition of pathogenicity is incomplete. The colon bacillus grows and multiplies within the animal body and yet it is, usually, a harmless inhabitant of the human intestines. An infectious disease does not alone result from the growth and multiplication of bacteria, but is conditioned by the parenteral digestion of proteins, which as foreign elements are introduced into the blood stream and tissues as a result of the invasion of the bacterial organism. The pathogenicity of a micro-organism, therefore, is a function of its ability to grow and multiply within the animal body, and of its activity in leading to the introduction of foreign proteins into the blood and tissues of the same. This definition comprises the great majority of micro-organisms, but does not cover completely the few instances where the micro-organism, like the tetanus, diphtheria, and dysentery bacillus, has the additional faculty of secreting a free toxin.

Pollen cannot grow nor multiply within the host. Its pathogenic action, therefore, must rest either on its faculty of leading to parenteral proteolysis, or on its faculty of producing a free toxin, or on both factors.
combined, and any endeavor to explain individual susceptibility to hay fever will have first to consider this mechanism of the pathogenic action of pollen. The analysis of all the possible forms of disposition leads us back to this problem.

Dunbar believes that the pollen contains a true toxin in the sense of Ehrlich, and his work in the last ten years has been chiefly devoted to demonstrating the correctness of this view. For upon this assumption rests his specific serum therapy, the passive immunization with pollantin (pollen antitoxin), which he considers a true antitoxin.

Dunbar prepares his “pollentoxin” by extraction of the finely pulverized pollen with 5 per cent. NaCl solution at 37° C. with addition of 0.5 per cent. phenol. The precipitate consists of empty pollen membranes and starch-rods and is without action. The supernatant opalescing solution contains the active principle, and is intensely active for subjects of hay fever, but is absolutely without effect upon normal persons. By precipitating with eight times the volume of absolute alcohol an extremely poisonous substance can be obtained which gives all typical protein reactions. This substance is a mixture of a great number of protein substances which can be separated by fractional precipitation into pseudoglobulin, euglobulins, and albumins. Only the albumins, which amount to about 16 per cent. of the total proteins, contain the toxin, whereas the globulin fraction is entirely inactive. A toxin, free from protein substance, could not be obtained. Most of Dunbar’s experiments were, however, performed with the total proteins obtained by alcohol precipitation from the saline pollen extract.

This “toxin” is thermostable. On heating it for one hour at 70° no change of activity can be observed, and even on heating a 1/1,000 solution to 120° for an hour some activity may still be retained. On treatment with alkalies and acids, with pepsin and trypsin, the activity is weakened, but is still strong enough to be demonstrated in the experiments.

The conjunctival and nasal instillation of one drop of a solution of 1/40,000 of the protein produces the characteristic subjective and objective symptoms in persons who are subject to hay fever, within a few minutes, but is without effect in normal persons.

Praussnitz, a pupil of Dunbar, and himself subject to hay fever, in experiments with pollen toxin performed on himself, observed the appearance of typical asthmatic attacks a few hours after the introduction of the “toxin” into the conjunctiva. On injection of a toxin solution into the scarified skin, a few circumscribed urticarial wheals appeared within 15 minutes. Dunbar also made subcutaneous injections of the pollen toxin, which will be described later, in detail.

Pollen extract is specific. Patients suffering from spring catarrh react only to the extract of grass pollen and not to the extract of ambrosia.
pollen. The physiological effect of the pollen poison corresponds completely to the clinical symptoms due to the pollen itself, and the pollen protein must, therefore, be considered as the active principle in the etiology of hay fever. So far we agree with Dunbar and Praussnitz. But does this prove that the pollen poison is a true toxin in the sense of Ehrlich?

The chief characteristics of a true toxin, according to Ehrlich’s definition, are the following:

1. A true toxin is effective toward all normal animals of the same species in about the same degree.
2. A toxin shows its characteristic poisonous effect in very small doses.
3. It is a very labile substance, easily destroyed by heating, by acids and alkalies.
4. It acts in the animal body only after a definite incubation time.
5. The action of the toxin is strictly specific.
6. The toxin leads in the animal body to the production of an antitoxin which neutralizes the toxin according to the law of multiple proportions.

Ad 1. Every true toxin (diphtheria, tetanus, dysentery toxin; abrin and ricin) exerts its poisonous action on all animals of the same species. The hay fever poison is apparently innocuous to the greater part of the human species, while a small part are intensely affected.

Ad 2. This condition is met with by the pollen poison which acts in doses of .001 mg. in most hay fever patients.

Ad 3. The pollen poison is not thermolabile like a true toxin, but is thermostable. Acids and alkalies which annihilate the action of a true toxin, do not destroy its activity.

Ad 4. The intoxication with pollen protein does not show any incubation time; the action is almost immediate.

Ad 5. The action of the pollen protein is completely specific. This statement is correct within the limits of a group reaction. But if we assume that the pollen poison is a true toxin, we should arrive at the necessary consequence that the pollen “toxin” of all the different plant species represents a uniform toxin of the same physiological action, a very remarkable property, incompatible with the specificity of all known true toxins in the plant and animal kingdom.

Ad 6. The curve of neutralization of toxin and antitoxin does not follow the law of multiple proportions, like the diphtheria and tetanus antitoxins. This law states that if a certain quantity of antitoxin protects against a definite minimal active dose, that a multiple of this quantity of antitoxin must afford protection against the same multiple of the minimal active dose. According to Praussnitz, who has performed these experiments, 2 toxin units would require 3.8 antitoxin units, but 5 toxin units require 125 antitoxin units and not 9.5 antitoxin units; if
the toxin units are further increased, such hundred and thousandfold quantities of serum would be necessary that the neutralization of these quantities in the conjunctiva would be an absolutely impossible thing. The neutralization curve of Dunbar's "toxin and antitoxin," therefore, does not even approximately follow the law of multiple proportions.

To this failure of meeting the characteristic requirements of a toxin, still other evidence is added in the behavior of the antitoxin, whose preparation and valuation will be discussed later. The pollen antitoxin, if applied subcutaneously, is poisonous and produces symptoms which are very similar to a subcutaneous injection of toxin. The immunity produced by a subcutaneous injection of even large doses of serum leads to a protection which lasts only for a few hours.

From all this evidence we reach the conclusion that: (1) The active principle of a pollen protein is not a toxin; (2) pollantin is not an antitoxic serum; (3) pathogenic action of the pollen cannot be explained on the assumption that the pollen contains a true toxin; (4) any immunity produced against hay fever cannot be an antitoxic immunity.

There remains thus only one other possibility of explaining the pathogenicity of the pollen and this is on the ground that the pollen protein acts as a sensitizer and as a poison by entering the circulation and tissues of individuals subject to the disease. This conception that hay fever results from the parenteral digestion of the proteins of pollen has been first suggested by Weichhardt and by Wolff-Eisner. Convincing evidence that the symptoms of pollen disease are called forth by the sensitizing and poisonous (anaphylatoxic) action of the pollen protein is brought out by the writer's own experimental work.

The specific skin reaction which hay fever patients show when an extract of pollen is applied to the scarified skin, or, as we proceed by intracutaneous injection, is best explained on the basis that the blood, tissues, and, therefore, also the skin, contain a specific proteolytic ferment which splits the pollen protein into a poisonous group that causes this inflammatory reaction.

The anaphylactic condition is further characterized by the possibility of transferring this state to another animal by injecting it with the serum of the sensitized animal (passive anaphylaxis). We used for this purpose serum taken in the third week of their seasonal attacks of two patients who suffered severely from asthmatic attacks. Four c. c. each of the serum of these two patients was injected intracardially into guinea-pigs. On reinjection, after 24 hours, all animals showed severe typical symptoms of anaphylactic shock.

No animal experiment can furnish as convincing an argument as the reaction of the human organism. It is a fact that hay fever is a condition of sensitization and anaphylaxis through the pollen protein, then the hay fever patient must be at any time of the year sensitized to the pollen.
protein and accordingly react with the typical symptoms of anaphylaxis on reinjection. This experiment Dunbar himself has performed on his assistant, Praussnitz, a subject of hay fever, and he gives an excellent description of all the symptoms of anaphylaxis characteristic for man, though, of course, he does not record it under this title. He writes: "The results obtained were of extraordinary interest, but at the same time terrifying. If a small amount of the toxin was injected subcutaneously in the forearm, the first manifestation appeared in ten minutes and consisted of severe sneezing with plentiful secretion from the nasal mucous membranes and considerable swelling of both nostrils; after 30 minutes a dry cough appeared with a slight tenacious expectoration, and at the same time the face swelled and became very red and cyanotic. A marked injection of the conjunctivæ developed and later chemosis. In both ears there was a feeling of tension objectively, however, no change could be perceived in the tympanic membranes. One hour after the injection tormenting asthmatic disorders with audible stridor arose; an hour later an urticaria-like eruption of large wheals appeared over the whole skin associated with violent itching; three hours after the injection the forearm began to swell. The edema spread during the following night to the whole arm. The edema of the arm, and a turgid appearance of the face, remained for several days. All other objective phenomena had disappeared by the next morning. The temperature remained normal from the beginning. Nothing abnormal was found in the urine. For a week after the experiment the patient experienced a disturbing sensation of weakness and exhaustion, as well as occasional attacks of palpitation of the heart. The same dose of pollen extract on a normal person not subject to hay fever produced only a slight, somewhat itching circumscribed edema around the place of injection."

We did not repeat this experiment, as we considered it too dangerous. But at the beginning of our endeavors to establish an active immunization in hay fever patients by the subcutaneous injection of pollen extract, we had one patient who reacted to the initial dose of 1 c.c. of pollen extract (1:10,000,000) with a slight attack of hay fever, which the patient himself diagnosed at once.

Guinea-pigs can be readily sensitized to pollen protein and succumb on reinjection with typical anaphylactic shock.

That the hay fever protein enters blood and tissue could further be shown by the demonstration of immune bodies in the blood serum of patients, while normal persons showed none. Specific precipitins could be demonstrated in four patients out of ten, while complement-fixing substances could be found seven times out of ten. It may be emphasized that it is very questionable if these substances are "protective" substances in the strict sense of the word, but, rather, reaction substances due to the introduction of foreign protein. Precipitin formation at least
seems to be a sign that the latter has brought about an injury of the tissue cells.

It is obvious that the most convincing proof that hay fever is a disease due to a specific protein anaphylaxis would lie in the actual demonstration of the foreign protein in the blood of the hay fever patient. On first thought this endeavor seemed futile, since the quantity of pollen protein which could reach the circulation would probably be too small to be demonstrable. But a simple calculation showed us that with the method of choice for identifying a specific protein, the anaphylactic reaction in the guinea-pig, the concentration of the protein in the blood could well be within the limits of experimental possibilities. At the time when the greatest amount of pollen is in the air, about 5 to 10 pollen grains are inhaled with each inhalation into the nasal cavity and about the same quantity reaches the conjunctivae and oral cavity in this time. Thus a person takes up about 10 to 20 pollen grains with each inspiration, which amounts to about 18,000 pollen grains per hour. A patient who spends about ten hours out-of-doors takes up pro diem pollen protein which equals 180,000 pollen grains. Since about 90,000,000 ragweed grains weigh 1 gm., 180,000 pollen grains are equal to .002 gm. of pollen; and on the basis that 40 per cent. of the pollen substance is protein, the actual protein introduced amounts to .0008 gm. Therefore, if we assume that a patient is exposed for three days to the same amount of pollen, it is possible that he has an amount of pollen protein in his blood equal to 540,000 pollen grains. Provided that the protein is not broken up thus rapidly into proteoses and amino-acids to prevent its demonstration by the anaphylactic experiment, this quantity would be sufficient for a trial experiment. For, if we figured the amount of blood of an individual with 160 lbs. weight to be 5 liters, we would reach the conclusion that every c. mm. of blood contains pollen protein equal to about 100 pollen grains. Since in subjects sensitive to hay fever all the characteristic symptoms of the disease can be produced by the nasal application of an amount of protein equal to 10 to 50 pollen grains, and this dose is necessarily much larger than the sensitizing dose, pollen protein could be present in the blood of patients in sufficient concentration. From J. B., a patient who had severe asthmatic attacks due to hay fever, which in one season lasted for four weeks, we obtained a sufficient amount of blood to yield 20 c. c. of clear serum. Four guinea-pigs were sensitized by the subcutaneous injection of 5 c. c. each, and reinjected after 12 to 18 days intracardially with 1 c. c. of a solution of 1/10,000 ragweed pollen extract. Three of the four animals showed severe typical symptoms of anaphylaxis, but recovered. Control animals were not affected by the same dose of pollen extract. We must, therefore, conclude that the guinea-pigs had been sensitized with pollen protein which was present in the serum.

It is thus absolutely proven by rigid experiments that hay fever is a
disease due to pollen protein sensitization and anaphylaxis and it is no longer permissible to speak of this established fact as a conception or an hypothesis.

On this basis not only do we comprehend the pathogenicity of the pollen protein and the mechanism of reaction, but also the "peculiar" individual disposition finds a satisfactory explanation. The pollen protein reaches at some season of the year the nasal mucous membranes of all persons. The human blood and all secretions of the mucous membranes of the body are digestive fluids and have normally very remarkable proteolytic properties. It has been known for a long time that the normal nasal secretion shows a decided bactericidal power; there is no doubt that this is largely due to proteolytic enzymes, whose presence has been experimentally established. This proteolytic enzyme of the nasal secretion in most people gradually splits the pollen like any other foreign protein, into harmless products, proteoses, and amino-acids. This cleavage proceeds slowly step by step just as the cleavage of the protein material in the stomach. The poisonous group contained in every protein molecule is, therefore, at any one time, present only in small concentration, and since its diffusibility is low, it is rendered inert as cleavage proceeds. The absorption of protein through the nasal mucous membrane is under normal conditions exceedingly minute, practically nil. Under various conditions, however, which interfere with the normal digestive function of the nasal mucosa, foreign protein, e.g., pollen protein, will be resorbed in sufficient amount to lead to sensitization. Conditions of this kind are: (1) a temporarily insufficient nasal secretion associated with a lowered quantity of normal proteolytic enzyme; (2) stenosis of the nasal canals through hypertrophied turbinates leading to excessive accumulation of inhaled matter and thus to increased resorption; (3) the accidental inhalation of an amount of pollen exceeding the protective function of the exposed mucous surface in the state of temporary nasal achylia mentioned under (1).

The protein absorption in the nasal cavity is easily comparable to the gastro-intestinal protein resorption. Normally only a minimal amount of genuine protein passes through the gastro-intestinal mucosa. If, however, the peptic and tryptic action is impaired, e.g., through pancreatectomy, or if a stenosis is produced by ligating off a part of the intestines, or if an overwhelming amount of protein is introduced, then a sufficient amount of genuine protein passes through the gastro-intestinal wall to be detected in the blood. (4) A factor, certainly only of subordinate importance, which might be considered by some as playing a rôle in the resorption of protein from the nasal mucosa, lies in the active proteolytic power, which most unicellular organisms possess and by means of which they might penetrate into body cells. The pollen grain possesses a special proteolytic enzyme, by whose activity the pollen is, in the process of fertilization,
enabled to penetrate the stigma ovule. But the resorption of unformed protein, devoid of this ferment action, similar to the resorption of egg albumen by the gastro-intestinal mucosa under similar circumstances, proves that this active proteolytic power of the pollen can well be neglected.

Whichever of the first three conditions named above brings about in a special case the resorption of pollen protein by the nasal mucosa, the all-important factor is, that this first parenteral intake of the foreign protein, through a process not perceived by the individual whose tissue takes it up, has a twofold effect: (1) It injures the epithelial cells of the mucosa and the endothelial cells of the finer capillaries in such a way that the mucosa remains permanently in a state of increased permeability for the protein (endothelial lysis); and (2) it brings about a new function of these cells, which consists in the production of a specific protective ferment directed against the specific pollen protein—the local tissue becomes sensitized.

The primary local sensitization which in a similar way can originate first in the conjunctivæ, does not remain localized, and the pollen protein gradually reaching every tissue of the body with the blood stream, causes all fixed tissue cells, especially those of the endothelial and epithelial systems, to develop this new proteolytic ferment against the pollen protein. This they pour forth, on renewed stimulation by the specific protein, on the surface of their layer or into the bloom stream. However, the cells and tissues first injured and sensitized retain this new specific proteoclastic function with greater persistence and in a higher degree than all other tissues; thus they remain in a sensitized stage and still continue to react when the blood fails to show any proteolytic action in the experiment in vitro.

So profound are these changes induced by the first absorption of the foreign protein that they may continue not only for one year, but for a lifetime, and may be transmitted from mother to child. The hay fever disposition—the pollen sensitization—can be and often is inherited.

When the same pollen protein which caused the sensitization reaches again the sensitized cells—in the flowering season of the next or any following year—the sensitized cells of the conjunctival and nasal mucosa pour forth the specific enzyme which readily attacks and dissolves the protein which in the state of solution can be readily absorbed.

The effects and symptoms evoked by this process depend largely on the speed with which the pollen protein is broken up, and thus on the concentration and quantity of the poisonous fraction (anaphylatoxin). The first symptoms are, therefore, local. The pollen reaching the mucosa of the conjunctiva, nose, and mouth, is split and the poison reaches the peripheral nerve endings, producing sneezing, itching, and burning in the eyes, nose, and palate, followed by vaso-dilation, hyperemia and inflammation on more extensive absorption. The increasing intra- and extra-
cellular activity leads to edema and exudation. The nasal respiration becomes impossible, and air, and with it the pollen, is inhaled through the mouth. The poison irritates the nerve endings of the palate and larynx, and itching of the palate and a dry laryngeal cough develop and through edema of the eustachian tube often a peculiar tense sensation in the ear.

With increasing concentration of the partly dissolved pollen protein more and more is resorbed into the tissues and blood and there is partly split into its poison. If the protein is set free in the superficial capillaries of the skin, localized areas of inflammation, acute edema with serous exudation will develop—urticaria. The whole organism shows the effects of the protein intoxication; if, preceding the hay fever attack, 3-hour temperatures have been taken and the normal curve of temperature has been established, elevations of 1° to 2°, alternating with hypothermia to the same extent, can be detected (protein fever).

The most dreaded symptom, the hay “asthma” or preferably pollen asthma, deserves special consideration in its relationship to the anaphylatoxic action of the pollen protein. The protein sensitization can account for this symptom in two different ways: (1) the pollen can reach the laryngeal and bronchial mucosa directly by inhalation and be split up in loco by the sensitized cells into the poisonous group; (2) the poisonous group reaches the bronchial mucosa and muscles by way of the circulation. The effect is the same: the anaphylatoxin produces diffuse edema, exudation of the mucosa, and spastic contraction of the bronchial muscles, expiratory dyspnea, acute transitory emphysema—asthma.

Thus the individual disposition to hay fever is to be explained as a pollen protein sensitization, which may have been inherited, or which may have been acquired during any period of life.

**SPECIFIC TREATMENT**

**Passive Immunization**

*Pollantin*

**Preparation of Dunbar's Antitoxic Immune Serum Pollantin.**—Only such horses as show a decisive reaction on a first subcutaneous injection of 0.5 gm. pollen or 0.2 pollen protein are considered suitable animals for immunization. The reactivity to this first injection varies greatly in different horses. Some show an urticaria with wheals the size of a walnut all over the body and a local swelling of 10 to 20 c. c. diameter at the place of injection, on the neck. In others this swelling takes the form of an edematous tumefaction of 50 to 75 c. c. in diameter and is associated with severe general symptoms as high fever, profuse perspiration, convulsive tremor, and loss of appetite. The symptoms are often so severe that
the animals seem near death; yet fatal intoxications do not occur at the first injection. But it is important to note that three out of nine horses showed a local susceptibility of the conjunctivae to a subsequent instillation of pollen extract.

The horses which react to the first injection of pollen receive, at intervals of several days, increasing doses of pollen extract. The maximum of the instillation is usually reached after three to four months' preparation, and can be kept at this same for months and years by occasionally re-injecting the animals. In the course of these injections the animals acquire a considerable tolerance against the poison; they can receive as much as 20 to 30 times the original dose without manifesting any symptoms. Dunbar states that the animals often furnish, after a preparatory treatment of several weeks, a serum which not only shows no protective action by neutralizing the pollen toxin, but even increases markedly the effect of the pollen toxin upon the hay fever patient. This action, which he attributes to not completely resorbed or not completely split pollen protein, disappears after several months of continued injection of the animals and the serum shows then decidedly protective properties. At the height of immunization, ascertained by measuring the potency of the serum in the manner to be described immediately, the animals are bled; to the clear separated serum 0.25 per cent. phenol is added, and it is kept either in a liquid condition or dried in vacuo at 45° C. The dried serum is ground to a fine powder and is mixed with one and one-half times its weight of pure lactose; this addition is supposed to decrease the irritative effect of the pure serum. The whole preparation must be carried out under carefully guarded aseptic conditions.

**Determination of the Antitoxic Value of the Serum.**—Since no animals can be found susceptible to pollen toxin, the potency of the serum is measured on hay fever patients by the ophthalmic reaction produced by a toxin antitoxin mixture. Very minute quantities of free pollen toxin produce hyperemia, congestion, and edema of the conjunctivae, and by spreading in a short time through the lacrimal channel to the nasal mucosa, attacks of sneezing. While most patients react markedly to a dose of 1/200 mg., very susceptible patients react to 1/40,000 mg. of pollen protein. These are quantities immeasurable with the most delicate quantitative scales and the sensitiveness of the reaction is far superior to the most delicate chemical tests.

Dunbar, after frequently repeated reaction tests, makes the very important statement that the same patient reacts to the same quantities of pollen toxin with absolute constancy and he is corroborated in this by his pupil Praussnitz, who recently stated that after carrying out thousands of such reactions on his own eyes, he has failed to observe any noticeable change in his susceptibility.

To determine the potency of the serum, the following method of Dun-
bar and Praussnitz is used. First the patient's susceptibility to pollen toxin is determined; i.e. that pollen toxin dilution which causes a decidedly marked opthalmic reaction. Then two sets of mixtures of the active toxin solution are prepared, the first with normal sera of different animals, and the second with varying quantities of immune serum. While the toxin solutions mixed with normal sera are not changed at all in their toxic effect upon the conjunctivæ, they are neutralized by a correct proportional quantity of immune serum. "In order to have definite results the toxin solution is used in double the necessary concentration. If the patient shows an objective reaction with a solution of 1–40,000, a solution of 1–10,000 is prepared. If the serum is supposed to be '40 fold' a dilution 1–20 is made. Equal parts of these solutions being mixed, the toxin is present in 20,000 fold, and the serum is 40 fold dilution. The mixture is kept for 30 minutes at 37° C. and then tested on the patient. If the serum is really 40 fold, no objective or subjective symptoms occur. If the patient feels a slight itching but no objective signs are observed, the serum is registered as 'bordering on 40 fold.' If objective symptoms are seen, the serum is next noted for 30 fold potency, and so on." The error of this method of estimating the potency of the serum is, in the hands of an experienced worker, not more than 10 per cent.

Only such sera as are still effective in a 30 to 40 fold dilution are put on the market. Exceptionally immune sera are obtained which are still effective in 60 to 70 fold dilution. By simultaneous or successive injection of different species of pollen, polyvalent sera can be obtained. The serum is stable and retains its antitoxic properties for one to two years if kept on ice. The powdered serum appears in this respect to be superior to the liquid. The antitoxic properties of the serum are fixed in the globulin fraction of the protein, as can be shown by fractional precipitation with ammonium sulphate.

Method of Use.—Dunbar's antitoxic serum is manufactured and patented under the name of pollantin. It is prepared in five different forms, all of which are intended for local use only.

1. Liquid pollantin. Antitoxic horse serum plus ¼ per cent. phenol. This preparation decomposes very readily, is easily contaminated and the carbolic odor is repulsive to many patients.

2. Pollantin powder. The antitoxic serum is dried in a vacuum apparatus, finely powdered, and three parts of lactose added to two parts of serum to decrease the irritative action of the pure serum.

3. Pollantin ointment. This preparation has in the hands of the writer given the best results since it combines the action of the serum with the mechanical action of the salve, which renders absorption more difficult.

4. Pollantin pastilles have been used successfully by several patients against the asthmatic symptoms (Dunbar).
SPECIFIC TREATMENT

5. Pollantin \textit{H}. is rabbits' anti-pollen serum especially designed for the use of those patients who are hypersusceptible to horse serum.

The pollen immune serum cannot be used subcutaneously or intravenously, for on account of the daily spontaneous reinoculation with pollen through the air, it would be necessary to give each day or every other day an injection of a rather large volume of horse serum. This is not only painful but not without danger, since it leads in some persons to serum disease, the symptoms of which are just as bad as those of hay fever. Therefore, pollantin is used only locally. The powder is to be preferred to the liquid, as it contains no phenol, and is more easily applied to the nasal cavity. The method of employment is very simple. With a fine brush minute quantities are placed in the conjunctival sacs, and with a powder insufflator blown into the nasal cavities. It is absolutely contraindicated to use too large quantities of the serum; it cannot be dissolved rapidly enough and irritates the mucous membranes as a foreign body. In some instances it renders people anaphylactic to horse serum. The size of a hempseed of powder (about 1 to 2 mg.) is sufficient for the nose and it is better to repeat the application instead of using a larger amount at one time.

If the eye symptoms are severe and conjunctival irritation troublesome, the liquid pollantin must be used. Against the tracheobronchial symptoms it is best applied with a spray. The treatment of the nose is the most important since the largest quantities of pollen are taken up in this organ. In order to obtain the best results it is essential at the hay fever period to use the serum every day, prophylactically, before the symptoms have appeared, because the edematous and congested mucous membrane has a very insufficient absorption power for the antitoxin.

Results of Treatment with Dunbar's Hay Fever Serum.—The results obtained with pollantin are somewhat contradictory. According to the experience of Dunbar, Luebbert (36), Praussnitz, Rosenberg, Zarniko (61), about 50 to 60 per cent. of all patients can be kept free from hay fever by the proper use of the serum. In about 25 per cent. of the cases a "partial" result is recorded, which includes such patients as felt subjectively a manifest improvement and whose suffering was shortened, but not completely relieved; only about 15 per cent. of the patients received no benefit from the treatment.

Similar figures are recorded by some American observers, as McCoy (38) and Somers (46), whose observations both appeared in 1903 under the influence of the first enthusiastic reports from Europe. The statistics of the German Hay Fever Association record a full success in about 25 to 35 per cent. of all cases, and a partial result in 25 per cent.; the remaining 40 to 50 per cent. of the patients obtained either no benefit, or state even an aggravation of their suffering. The Secretary's report of the United States Hay Fever Association for the year 1909 contains references
to twenty cases treated with pollantin; of these eight were relieved and twelve not relieved. Most of those who reported relief from pollantin stated that after it had been used for a time, it ceased to be effective.

My own experience with pollantin is limited, since I have employed active immunization in the last four years, almost exclusively. Only on patients who refuse to have subcutaneous injections, or who come very late in the season, do I use pollantin. In a few cases I have used the combined treatment of active and passive immunization. Judging by this limited experience with pollantin, which comprises about twenty patients, I should say that pollantin relieves the eye and nose symptoms in cases of slight severity, but is of no effect in alleviating the tracheobronchial symptoms, cough and asthma. Some patients, on whom I wanted to use pollantin combined with active immunization, refused to have it, as they were convinced that it made them worse, and in two patients suffering from a severe type of the disease, I could confirm this statement.

Such observations, that the symptoms of hay fever are in some patients intensified by the use of pollantin, have been observed repeatedly here and abroad. Dunbar explains all these cases as due to anaphylaxis to horse serum which the patients acquired by the prolonged use of the serum, especially if applied in too large doses. I have convinced myself that this does not hold good for all cases. Four patients who showed this intolerance for pollantin reacted with the same intensity toward a rabbit anti-pollen serum, which I had prepared in the Memorial Institute for Infectious Diseases. It is very improbable that these four patients should all have been hypersusceptible to rabbit serum too, as they had never before received it. I am inclined to see the harmful effect of pollantin in such cases are due to the specific proteolytic power of the serum which, on activation by the patient’s complement, acts with increased speed and in this way increases the concentration and quantity of pollen poison. That pollantin does not exercise this harmful effect in all patients is due to the age of the serum, which is practically never used in a fresh condition, and to the fact that not all patients contain the necessary substances in sufficient amount to activate the serum.

**Graminol**

In the paragraph on pathogenesis and immunity, the reasons are given why the pollen poison cannot be considered as a true toxin and why pollantin is not an antitoxic serum. The beneficial effect which pollantin displays in hay fever of moderate severity cannot lie in any specific antitoxic action of the serum. Very soon after Dunbar’s pollantin appeared, another hay fever serum, “graminol,” was prepared by Weichhardt and put on the market. This serum is not claimed to be an antitoxic pollen serum, but a normal serum obtained from cattle during the time of the flowering
of grasses. It is claimed by Weichhardt that this serum contains immune bodies which, though present in beef serum during the whole year, are especially plentiful at the time when the cattle feed on the flowering grasses. By a special process this serum is concentrated and its potency increased. The theoretical foundations for the activity of graminol are exceedingly weak. It has been shown that pollen protein does not penetrate from the gastro-intestinal canal into the circulation; nor has graminol any antitoxic properties whatsoever. I was not able to demonstrate in it any specific antibodies as precipitins or complement-fixing antibodies against timothy pollen extracts. We therefore reach the conclusion that graminol is normal beef serum. Yet the results obtained with it in the treatment of hay fever are surprising and startling.

The Reports of the German Hay Fever Associations show that the results obtained with graminol are certainly not inferior to those obtained with pollantin.

<table>
<thead>
<tr>
<th>Year</th>
<th>Serum Used</th>
<th>Successful Per Cent.</th>
<th>No Success Per Cent.</th>
<th>Secondary Effects from the Serum Per Cent.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1906</td>
<td>Pollantin</td>
<td>69</td>
<td>31</td>
<td>22</td>
</tr>
<tr>
<td>1906</td>
<td>Graminol</td>
<td>75</td>
<td>25</td>
<td>14</td>
</tr>
<tr>
<td>1907</td>
<td>Pollantin</td>
<td>59</td>
<td>27</td>
<td>14</td>
</tr>
<tr>
<td>1907</td>
<td>Graminol</td>
<td>61</td>
<td>35</td>
<td>5</td>
</tr>
</tbody>
</table>

These finding are corroborated by Wolff-Eisner (58) who obtained better results with graminol than with pollantin. Since graminol is nothing more nor less than normal beef serum and its action in alleviating the symptoms of hay fever is not different in any respect from pollantin, we are led to the conclusion that the beneficial effect of pollantin is also due to the same non-specific properties in the horse serum.

Normal serum contains not only proteolytic ferment, but also various antagonistic substances, antiproteolytic ferments, which if more effective than the former, can counteract to some extent the specific proteolytic action of the patients' serum. Since pollantin by the mode of its preparation is richer in proteolytic substances than graminol, therefore richer in anti-ferments, the superior effect of the latter may lie in this higher anti-ferment concentration. (Wolff-Eisner.) Another explanation of the action of pollantin and graminol seems possible to us. The poisonous effect of the anaphylatoxin leads temporarily to morphological and functional changes in the tissues and blood, which might be considered as a true state of
transient hemophilia. The whole endothelial apparatus is injured; the capillaries show an increased permeability, there is an abundant exudation of serum and increased incoagulability of the blood. The frequent application of the pollantin and graminol sera supplies to the tissues primarily involved thrombosin and other thromboplastic substances of colloidal character. They form fine precipitates and films with the patient's blood, increase the viscosity and coagulability, and thus temporarily decrease resorption of the pollen protein.

Since pollantin, like any antiserum, is richer in antithrombosin, and poorer in thrombosin, it would be inferior in its action to graminol. The therapeutic effect of normal serum in urticaria, a characteristic symptom of anaphylaxis in man, has recently been reported by Linser. We thus reach the conclusion that pollantin does not benefit hay fever patients by an antitoxic or other specific action; the only specific action which it possesses—that of facilitating the local breakdown of the specific pollen protein—can be regarded only as a disadvantage.

**Active Immunization**

The clinical observation that the susceptibility to hay fever decreases, in some patients, with increasing years suggests that an increased tolerance may be acquired during life through the annual inoculations applied by nature. That this immunization is not accomplished oftener is probably due to the capricious method of these inoculations, which are applied several times a day for many weeks and must lead only to the exhaustion of any protective substances formed. The conception of pollen disease as a protein sensitization primarily localized in certain tissues suggested to us the possibility of establishing this higher tolerance for pollen protein by increasing the area of sensitization by subcutaneous injection of pollen extracts.

What might be considered the first endeavor to produce an active immunity against hay fever was reported in 1900 by Holbrook Curtis (17). He used weak extracts of the whole plant (ragweed and goldenrod) in subcutaneous injections and reports favorable results with this treatment. Wagner (52), Ingals (33) and others have also used various plant extracts for the same purpose and speak favorably of this treatment. Still no mention is made of any continuation of these first trials of active immunization. Dunbar, at the beginning of his researches on hay fever, had given subcutaneous injections of pollen extracts, using doses which, in the present light of our knowledge, were excessive—being about 100,000 times that which is at the present time our average initial dose. He obtained such terrifying results that he did not proceed further along this line of treatment.

In 1911, Noon (39), and Freeman (27) published from Wright's
laboratory in London, the first scientifically conducted and controlled experiments on active immunization in hay fever by hypodermic inoculation with pollen vaccine. Noon injected several patients during fall, winter and spring, with increasing doses of pollen extract of timothy. The interval between the injections varied from 3 to 14 days. The results obtained were controlled by testing the susceptibility of the conjunctiva before and after the injection, and it was found that it decreased markedly as the treatment proceeded. If too large doses were used, the susceptibility was found to increase again but soon fell below the value first observed. In this way Noon succeeded in decreasing the susceptibility to 1/100 in most of his patients. Freeman continued the experiments of Noon on the same and on new patients up to and during the critical season. He treated in all 18 patients. Of these 10 had received the treatment prophylactically during winter and spring; 8 after the symptoms of hay fever had developed. He, too, succeeded in reducing the susceptibility of the conjunctiva usually to 1/10; in two cases to 1/100 of the original value. Three patients obtained excellent results; 13 were considerably improved; 2 patients failed to receive any benefit from the treatment.

In the course of a study on the pathogenesis of bronchial asthma and its treatment by means of bacterial vaccines, which dates back to 1908, I became interested in hay fever. In May, 1910, unaware of the work on this subject done in A. E. Wright's laboratory, I began active immunization against hay fever, and thus far I have treated forty-one patients by this method. Thirty-six of these patients suffered from autumnal catarrh and only two from the spring variety, studied by Noon and Freeman.

A preliminary communication on work along the same line was published last year by George H. Clowes (16), who reported favorably on the treatment of eight cases.

The Preparation of Pollen Extract.—The author's technique, employed in obtaining efficient pollen extract, consists in the following procedures:

Saline Extract.—0.1 gm. (1 cg.) of pollen is broken up as finely as possible in an agate mortar and gradually 10 c. c. of a 8.5 per cent. salt solution, ten times as strong as a physiological salt solution is added drop by drop. This saline suspension is shaken for 2 hours and then left in the incubator at 37° C. for 16 hours. Then the extract is again shaken for 2 hours, centrifuged, and the supernatant fluid separated with a pipette from the undissolved residue. The supernatant fluid which is a dilution of 1/1000, is diluted ten times with sterile distilled water plus .25 per cent. phenol, which makes the salt solution a physiological one and the dilution 1/100,000. From this dilution all others are prepared. This dilution and all lower ones are unstable and deteriorate by progressive proteolysis into a toxic product within 8 to 10 days. The concentrated pollen solu-
tion in 8.5 per cent. saline is more stable and on ice, can be kept for three weeks.

Alcoholic Extract.—The pollen is first extracted with 8.5 per cent. saline solution and the whole procedure carried on as in saline extract. But the supernatant saline solution after dilution with water to 0.86 per cent. NaCl content is precipitated with 10 times its volume of 95 per cent. alcohol. The yellowish-white precipitate is filtered off and dried in vacuo to a hard mass. This mass yields a yellowish-white powder, which in a dilution of 1–100,000 produces the typical reaction if instilled into the conjunctiva.

Vaughan’s Method.—We tried further by Vaughan’s method (2 per cent. NaOH in absolute alcohol) to disrupt the pollen protein in the endeavor to obtain a pollen protein free from poisonous properties. Our experience with this preparation is too limited to be referred to here in more detail.

Experiments showed that all patients subject to autumnal catarrh react most strongly to ragweed pollen extract and we, therefore, used this extract exclusively. The initial dose was determined in the following manner:

We first established in every patient the minimal toxic dose as determined by the instillation of one drop of pollen extract into the conjunctival sac. This means the highest possible dilution, which in the quantity of one drop (1/20 c.c.) still produces an objectively marked hyperemia. The strength of pollen extract required to bring about this objectively marked hyperemia varies in different patients. In those who have not been actively immunized before, it lies between 1/20 c.c. of a dilution of 1–500,000 to 1/20 c.c. of a dilution of 1–10,000; or, expressed in actual quantities of the soluble pollen protein, between 1/10,000,000 to 1/200,000 of one gram. This ophthalmic reaction at the same time serves diagnostic purposes, and has been advocated for years by Dunbar. Patients who do not suffer from pollen disease do not give this test, even on using ten thousand times as large quantities of protein. We tried first to use the intracutaneous test for determining the individual resistance of the patients, but we could not obtain satisfactory quantitative results. The susceptibility of the patient, determined by the strength of the pollen extract which in the quantity of 1/20 c.c., i. e., one drop, causes hyperemia of the eye, is the indicator which determines the initial dose to be used. Noon and Freeman, who proceeded in a similar manner, took as initial dose 1/3 c.c. of that dilution of which one drop produced the eye reaction. But the pollen extract of the composite, and especially ragweed, is far more toxic than that from graminaceae. We therefore use one-half of the actual quantity of pollen protein which gives a characteristic ophthalmic reaction for the initial immunizing dose. Thus, if a patient reacted to 1/20 of a dilution 1–500,000, which equals 1/10,000,000 of one gram of soluble pollen protein, we start with a dose which is equal to
1/20,000,000 of one gram of pollen protein. Under no circumstances, however, do we use an initial dose larger than 1 c.c. of a dilution 1–1,000,000; i.e., 0.000,001 gm. pollen protein even if the ophthalmic reaction would have indicated, according to the above calculation, a stronger dose. For instance, if a patient reacts only to 1/20 c.c. (one drop of a dilution 1–10,000), i.e., to 0.000,005 gr. of soluble pollen protein, we do not use 1/2 c.c. of a dilution of 1–200,000, 0.000,002.5 gr. of pollen protein, which would be one-half of the quantity which gives the eye reaction, but we start immunization with the maximum initial dose, which we consider permissible, 1 c.c. of a 1–1,000,000 dilution. To facilitate expression and a rapid comparison between the doses used for testing the susceptibility of the patient and the doses used for immunization, we have adopted a unit of pollen toxin. We understand as the unit of pollen toxin the 1/100 part of a millionth of a gram of pollen protein and designate it as one P. U. = 0.000,000,01 gm., or, written in fraction, 1/100,000,000 of one gm. Thus if a patient who is very sensitive to pollen protein gave an objectively marked ophthalmic reaction to one drop (1/20 c.c. of a solution 1–500,000), we should have in this 1/20 c.c. 1/10,000,000 of one gram of soluble pollen protein, and this resistance would be designated as R = 10 U. P. We should begin his immunization with 1/20,000,000 of a gram of soluble protein: for instance, 1 c.c. of a solution 1–20,000,000, and say the immunizing dose is 5 U. P.

The initial dose is determined in the way described. The subsequent doses, the intervals of time to be observed, and the frequency depend altogether on the time at which the patient undergoes the treatment. It is most to be desired that the treatment be a prophylactic one, though we have obtained very encouraging results with active immunization if started when the disease was already well developed. Since the first symptoms of autumnal catarrh begin between the 10th and 20th of August, the patients are advised to begin the prophylactic treatment in the first week of May. The resistance is determined by the ophthalmic reaction, and the first immunizing dose, determined as stated, is injected subcutaneously two or three days after the test was made. Only a very small number of patients show any local reaction at the site of injection, consisting in as light, reddish-colored tumefaction. The next injections are given at intervals of four to ten days, the smaller doses in shorter intervals, the larger one in longer. As far as the strength of the dose is concerned, their increase must be carried out according to the only method to be employed in the active immunization of any disease, this principle, laid down by Koessler and Neuman (34), in a study on tuberculin treatment some years ago, might be expressed as follows: The increase of doses in active immunization must proceed in such a way that there exists not only an actual increase of dosage, but the difference between the next following and the preceding doses must continually increase or remain the
same. But an increase by geometric progression can take place only in the first two or three injections, as this would increase the dosage so rapidly that the patient would respond with very severe reactions. These overdoses not only do not immunize, but they lower the resistance very considerably. To illustrate a possible route of immunization with pollen extract. Suppose the ophthalmic reaction was produced with $1/10,000,000$ of one gram of soluble protein contained in $1/20$ c.c. (one drop) $= 10$ U. P., then the initial immunizing dose would be $5$ U. P., i.e., $1/20,000$ of one gram of soluble pollen protein contained in $1$ c.c. or $1$ c.c. of a dilution $1-20,000,000$. The subsequent doses would be as follows:

<table>
<thead>
<tr>
<th>U. P.</th>
<th>Gm.</th>
<th>C. C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>1/20,000,000</td>
<td>.5 c.c. of a dilution 1-10,000,000</td>
</tr>
<tr>
<td>10</td>
<td>1/10,000,000</td>
<td>1.0 &quot; &quot; &quot; 1-10,000,000</td>
</tr>
<tr>
<td>20</td>
<td>3/20,000,000</td>
<td>.75 &quot; &quot; &quot; 1-5,000,000</td>
</tr>
<tr>
<td>15</td>
<td>1/5,000,000</td>
<td>1.0 &quot; &quot; &quot; 1-5,000,000</td>
</tr>
<tr>
<td>30</td>
<td>3/10,000,000</td>
<td>.6 &quot; &quot; &quot; 1-2,000,000</td>
</tr>
<tr>
<td>50</td>
<td>1/2,000,000</td>
<td>1.0 &quot; &quot; &quot; 1-2,000,000</td>
</tr>
<tr>
<td>75</td>
<td>3/4,000,000</td>
<td>.75 &quot; &quot; &quot; 1-1,000,000</td>
</tr>
<tr>
<td>100</td>
<td>1/1,000,000</td>
<td>1.0 &quot; &quot; &quot; 1-1,000,000</td>
</tr>
<tr>
<td>150</td>
<td>3/2,000,000</td>
<td>.6 &quot; &quot; &quot; 1-400,000</td>
</tr>
<tr>
<td>250</td>
<td>1/400,000</td>
<td>1.0 &quot; &quot; &quot; 1-400,000</td>
</tr>
<tr>
<td>375</td>
<td>3/800,000</td>
<td>.75 &quot; &quot; &quot; 1-200,000</td>
</tr>
<tr>
<td>500</td>
<td>1/200,000</td>
<td>1.0 &quot; &quot; &quot; 1-200,000</td>
</tr>
<tr>
<td>750</td>
<td>3/400,000</td>
<td>.75 &quot; &quot; &quot; 1-100,000</td>
</tr>
<tr>
<td>1000</td>
<td>1/100,000</td>
<td>1.0 &quot; &quot; &quot; 1-100,000</td>
</tr>
</tbody>
</table>

As seen from the table, the whole immunization demands only seven different dilutions. The injections are given in intervals of from 3 to 10
days, the smaller doses in shorter intervals, the larger at longer intervals. The immunizing effect of the injections is seen in the increased tolerance established when tested by the eye reaction. These tests are made every two or three weeks during the treatment. The tolerance rises steadily to a certain point, but later in the treatment the rise is slower and sometimes, a certain height reached, remains stationary. In a series of 20 patients the effect of every injection was controlled by the ophthalmic reaction in the endeavor to use it as a constant guide in determining the subsequent dose to be given. This has not only been found unnecessary, but even misleading in some respects. If carried out in the progressive way illustrated above, much less faulty doses are given than when based on the indications gathered from the ophthalmic reaction.

The conjunctival reaction teaches us that there is actually an increase in resistance produced through the treatment. Indeed after 8 to 10 subcutaneous injections the resistance in some patients, tested by the ophthalmic reaction, is raised from 40 U. P. to 600 U. P. If now the immunizing dose should be correspondingly increased, this increase would involve such a jump in dosage that it would unavoidably lead to severe reactions and the progress made by the treatment of several weeks would be undone by this faulty procedure. The resistance of the patient might even have reached a height so great that it would be impossible to increase any further the strength of the subcutaneous injection, and the resistance would only be further increased by the repetition of the same dose. Thus the ophthalmic reaction of one patient showed that in several months' treatment his resistance had increased from 10 U. P. to 6,000 U. P. This means that a patient who at the beginning of the treatment showed an ophthalmic reaction on the instillation of a drop of a dilution 1–500,000, reacted after nearly three months' treatment only to a drop of 1–800. It would have been impossible to increase the last immunizing dose, 1 c.c. of a solution 1–50,000, for this is the highest dose which is still well borne by most patients. As a rule, it is advisable not to go higher than 1 c.c. of a dilution 1–100,000, if unpleasant reactions are to be avoided. In some patients again, the ophthalmic resistance seems to have reached a certain maximum after several weeks treatment; several injections of gradually increasing doses do not bring about any change at all; it seems to remain stationary. But if we proceed carefully with the injections, the ophthalmic reaction shows suddenly a remarkable jump in resistance, and thus proves that we have increased the tolerance.

Though the eye reaction is not a safe index for determining the strength of the next dose to be used, it is, if tests are made repeatedly during and after the treatment, an excellent proof that the immunity of the patient against pollen protein has actually been increased by the injections. In some patients this increase has been several hundredfold and in a few instances even one thousandfold.
Increased resistance of the patient, measured by the ophthalmic reaction will be noted if the treatment is given not prophylactically, during health, but after the disease has fully developed. The understanding of the mechanism of vaccination during the diseased state is a very difficult problem, especially in bacterial diseases where it seems unreasonable to add to the milliards of virulent bacteria a few million dead ones. In hay fever the invasion of the mucous membranes with pollen leads, as we can show by the complement-fixation reaction, to an exhaustion of antibodies in the blood. It is not only possible, but very probable that these protective ferments have not disappeared suddenly from the blood, but that they have accumulated in the organs and tissues immediately affected. For the resistance of immunized patients tested by the eye reaction at a time when we were unable to demonstrate any antibodies in the blood did not show any considerable decrease. Such an antibody fixation in vivo is by no means an uncommon thing but is a far more general biological phenomena than is commonly appreciated. For instance, in echinococcus disease, the demonstration of complement-fixing antibodies enabled us in a good number of cases to make the correct diagnosis before the operation. There occurred, however, some instances where the test was negative and yet an echinococcus cyst was found. The cyst was removed as a whole, completely intact. A renewed test of the blood after several days was positive and showed a high concentration of antibodies. It is probable that the removal of the antigen disrupted the cell fixation of these protective substances; which, liberated, reached all organs of the organism and could be detected in the blood. So it does not seem absurd in pollen disease that, while the organs and tissues immediately affected are actually saturated with antibodies, other areas and cell territories completely impoverished, respond well and rapidly to a renewed injection of the sensitizer with an abundant production of protective substances. The duration of the resistance, increased in such a remarkable degree by active immunization, does not last equally long in all patients. We observed in the fall and winter of 1911-1912, that the susceptibility of patients treated in 1911 rose again rapidly during and after the hay fever season, as repeated ophthalmic tests proved. But 2 out of 10 patients retained a heightened resistance during the winter. Yet all patients who returned in the following May, 1912, responded with such promptness to the first three injections that a level of resistance was reached which in the previous year had not been accomplished at all in some patients, or in others only after months of treatment. This emphasized the fact that patients must return each season for several years if the treatment by active immunization is to have a permanent influence on their susceptibility until a level of resistance is reached which indicates a safeguard for any further invasion of pollen.

In the last four years I have treated 41 hay fever patients by active
immunization with pollen extracts. Five of these had the early spring
variety of the disease, and all these began treatment after the disease
was already developed. The remaining 36 had autumnal catarrh. Seventeen
of these patients had prophylactic treatment, whereas 19 were treated
while symptoms of hay fever were present. Four of these patients have
been completely free from hay fever, though remaining in their usual
place of abode. Of these four patients, three had treatment during two
years and one during three years, for two to three months. The supreme
test in patients who are apparently free from the disease is a railway or
an automobile journey through flowering meadows. Only these 4 patients
of the 41 could stand this experiment. Two of them have so far remained
free for two years, and two for one year.

Of the other 37 patients, 29 were markedly improved subjectively
as well as objectively; the remaining 8 must be considered objectively not
improved, though 5 of them feel subjectively benefited. The marked im-
provement consists in later, milder, and shorter attacks, in the possibility
of remaining in town and at work for the first time in years, in a dimin-
ution or disappearance of troublesome cough and constitutional symptoms.
Twenty-three of the 41 patients previously had asthmatic attacks during
the day and night in the critical season. Of these, 16 experienced an un-
doubted amelioration of this most distressing feature of the disease. Six
of these hay fever asthmatics had no asthmatic symptoms, though 5 of them
still retained other symptoms of hay fever. Others had only two or three
attacks during the whole season, were little disturbed in their sleep during
the night, while all emphasized the improvement experienced.

In conclusion, I wish to express emphatically a word of caution. It
will not be long before the commercial manufacturers of vaccines see "the
great advantage and benefit" of this treatment. Hay fever vaccines will
be praised and advertised and put up so attractively that their use will
become universal, and soon—universally discredited. For the pollen ex-
tract is not stable, especially not the higher dilutions. By progressing
proteolysis, after three to four weeks, it acquires marked toxic properties
which lead to severe reactions. The solutions must, therefore, be freshly
prepared every 8 to 10 days if these reactions are to be avoided. What-
ever the method of active immunization, whatever the dosage and tech-
nique, the one sound basis that must underlie all these endeavors is that
the material to be injected must be not only sterile, but constantly of uni-
form potency if used in the same dilution. No extract of pollen can com-
ply with this demand if it is older than three weeks.
REFERENCES

1. Beard, G. M. Hay Fever or Summer Catarrh, New York, 1876.
2. Binningerus, J. N., Observations et Curationum medicinalium cen-
turiae V. Montisbeligardi, 1673.
3. Binz, C. Pharmazeutische Studien über Chinin, Virchow’s Arch.,
   1869, xlvi, 101.
4. ———. Heusieber, Virchow’s Arch., 1871, li, 176.
5. Blackley, Ch. H. Experimental Researches on the Cause and Nature
6. ———. Bemerkungen über Dr. G. T. Patton’s Experiments über
   Heusieber, Virchow’s Arch., 1877, lxx.
7. ———. Hay Fever, Its Causes, Treatment and Effective Prevention,
   London, 1880.
8. ———. On the Treatment and Prevention of Hay Fever, Lancet,
   1881, ii, 371.
    437.
15. Cazenave, J. J. Observations de maladies périodiques, Gaz. Méd. de
    Paris, 1837, 630.
16. Clowes, G. H. A. A Preliminary Communication on the Treatment
    Biol. and Med., 1913, x, 70.
18. De Rebexque, C. J. Atrium Medicinae Helvetiorum, Obs. 92, Genevæ,
    1691.
    Heusiebers, München, 1903.
20. ———. Neuere experimentelle u. kritische Beiträge zur Heusieber-
23. ———. Neber das Serobiologische Verhalten der Geschlechtszellen.
    Zeitsch. f. Immun., 1910, iv, 740; and 1911, vii, 454.
24. Dunglison, R. Practice of Medicine, Philadelphia, 1842, 277.
25. Elliotson, J. Hay Fever, Lond. Med. Gaz., 1831, viii, 411; and
    Lancet, 1830-1831, ii, 370.
26. ——. Principles and Practice of Medicine, Phil., 1844, 771.
30. Helmholtz, H. See Binz, Virchow Arch., 1869, 46, 100.
35. Ledelius, S. Odor rosarum visui nocivus Miscellanea curiosa Decuriae II. Norimberge, 1684 and 1691.
43. ——. Heufieber Antitoxin, Ibid., ii, 263.
44. Riedlin, V. Linearum Medicarum anni Augustae Vindelicorum, 1695.
45. Schneider, K. V. De Catarrhosorum dieta et de specibus catarhorum libri v. Witteberge, 1662.
47. Sticker, G. Der Bostocksche Sommer Catarrh, Nothnagels Path. u. Therap., Vienna, 1896, 4, i.
48. ——. Das Heufieber, Wien., 1912.
SPECIFIC TREATMENT OF HAY FEVER

55. ——. Zur Serum behandlung des Heusiebers, Berl. klin. Woch., 1906, xliii, 118.
59. Wood, G. B. Practice of Medicine, Phil., 1849, 753.
60. Wyman, M. Autumnal Catarrh, N. Y., 1872.
CHAPTER XXXIII

HYDROPHOBIA

Anna Wessels Williams

INTRODUCTION

A knowledge of the treatment of hydrophobia includes a knowledge of the disease as it occurs in lower animals, as well as in man. The special prophylactic treatment, which consists in a series of daily inoculations of a specific vaccine, is comparatively long, uncomfortable, and expensive, therefore the unnecessary administration of it means more perhaps than in the treatment of many other diseases. In order to determine the wisdom of treatment in any case we must be able not only correctly to diagnose the presence or absence of the disease in the animal through which the infection was supposed to be transmitted, but to know the possibilities in cases that can only be called suspicious.

Though the incidence of hydrophobia in man is very small compared with that of other fatal affections, the disease is so dreaded, and its results are so terrible, that it needs to be thoroughly understood in order to be able, not only to know and handle it when it does appear, but also to advocate strongly the comparatively simple preventive measures against rabies in the dog which have been shown in certain parts of the world to be so efficacious.

DEFINITION AND SYNONYMS

Hydrophobia is an acute specific infectious disease of mammals, communicated usually by the bite of an infected animal (chiefly a canine), less frequently by the introduction into a recent wound of the specific virus through contact with the saliva of an infected animal.

It is characterized (1) by a long and variable incubation; (2) by the extremely short course and practically invariable mortality when symptoms develop; (3) by the localization of the virus chiefly in the central nervous system and the salivary glands; (4) by specific pathologic changes in the central nervous system; (5) by symptoms referable to these patho-
logic changes, i. e., first, symptoms of excitation, which may be most pronounced (furious hydrophobia), and, second, those of degeneration, which may be most pronounced (dumb hydrophobia); (6) by protection through inoculation with rabies vaccine.

The earliest known name for the disease is lyssa, which is a Greek word meaning madness. Celsus, in the first century, gave the name hydrophobia to the disease in man because of the frequent symptom of fear of water exhibited by man alone. Of course, as the disease has the same etiology in all animals, the name in all should be the same. The Romans called the disease rabies, meaning furious or raging, or aquafuga, meaning fear of water. The English call the disease either rabies or hydrophobia, and also speak of "mad animals." In Germany they say Wassercheu, Hundswut, Tollwut, or simply Wut; in France it is called la rage; in Italy rabbia; in Spain rabia or hydrophore.

Lyssophobia is the term used to designate the condition caused by fear of rabies after a non-infected bite. This, of course, is never by itself fatal.

HISTORY

The earliest written records of rabies are said to be found in the writings of Aristotle (about 300 B.C.). In them the statement is made that dogs are subject to rabies, and, when infected, communicate the disease by biting all other animals except man. Human rabies was not described in writing until the first century A.D., when Celsus gave what was evidently a compilation; hence the disease in humans must have been known before.

The paralytic form of the disease in dogs was first noted in 1714, and in human beings in 1753. The virulence of the saliva of dogs was shown in 1804, and Gruner (1813) recommended the inoculation of test animals with the saliva of suspicious dogs to determine diagnosis. In 1821 Magendie and Breschet stated that they transmitted the disease from man to dogs by saliva.

The history of rabies perhaps more than that of any other disease shows how the imagination of people may run wild through lack of knowledge. Though, from time to time, an investigator appeared who showed that he was able to make some true observations, the majority of writers uttered much superstitious nonsense as regards both the origin and the treatment of rabies. For example, some said it was caused by evil spirits and cured by pilgrimages; others that it was caused by fright and cured by self-control; still others claimed that it was caused by a lack of water and cured by very rough sea-bathing, and so on. Even the better observers often recommended empiric and bizarre remedies.

Though the rational treatment by the cauterization of the wounds was
among the earliest methods practiced, Razes and others advised keeping
the wounds open and suppurating for two months, and Galen recom-
mended extirpating the part bitten when possible.

Even now some people believe that dogs develop rabies because of
lack of water, others think that "mad stones" (calculi from the alimen-
tary tract of lower animals) cure, and still others think that there is no
such disease—that deaths are due to fright or to something else. Even
as late as 1900 the United States Government published a circular
through Dr. Salmon, Chief of the Bureau of Animal Industry, giving
facts in regard to the reality of rabies, stating that this pamphlet was
called forth by the opposition and disbelief expressed by people in letters
to the daily press which fostered and encouraged them at the same time by
editorials. This disbelief on the part of the people is due partly to a reac-
tion against the extravagant ideas that earlier prevailed, partly to a senti-
mentality that refuses to believe so bad a thing of this friend the dog, and
partly to the fact that so few people are bitten by mad dogs, and these
with so long an incubation and, even without treatment, such a low rate
of mortality after bites.

Though we do not yet know the full nature of the cause of rabies, we
know more about its etiology than we do about that of several other dis-
ese whose entities are accepted without question. In fact the specificity
of rabies has long been proved. Therefore the scepticism which still
exists in regard to it is entirely without foundation.

The most brilliant series of experiments to prove the entity of the
disease were carried on by Pasteur in the latter part of the nineteenth
century. As a result of the first part of his studies he made the an-
nouncement to the French Academy in 1884 that he was able to immunize
animals against rabies. The principle of his treatment was the same
as that demonstrated by him for anthrax, and much earlier by Jenner for
smallpox; that is, the production of immunity by inoculations of
an attenuated virus. Pasteur's continued studies in collaboration
with others and the essential details of his later method, as well as the
modifications tried by others, will be given under the heading
Treatment.

In the meantime the many efforts made to discover the cause of the
disease remained unavailing. It is true numerous authors from Pasteur
down described minute pleomorphic granules in the nerve tissue which
they said might be the specific micro-organisms, but no growths were ob-
tained, neither was other evidence forthcoming as to the parasitic nature of
these granules. That the cerebrospinal canal with its nerve tissue con-
tents is practically a test tube with living nerve cells as a medium in
which the rabies virus grows helped to solve the question of the specificity
of the virus, but did not demonstrate its nature.

Through these studies, however, some facts were learned in regard
to the nature of the virus, and in regard to certain microscopic appearances in the central nervous system.

Three of the histologic findings have been made use of in diagnosis: (1) "the rabic tubercles" of Babes; (2) "the areas of spheroidal and oval-celled infiltration" of Van Gehuchten and Nelis; and (3) and most important, the cell inclusions commonly known as Negri bodies, so called after Negri, who was the first (1903) to announce their discovery.

A number of other observers were studying the Negri bodies at the time of Negri’s announcement, and many have studied them since, without arriving at an agreement as to their nature. Practically all have come to the conclusion, however, that they are specific for rabies, and so are of great worth in diagnosis (see pp. 710 and 715).

GEOGRAPHICAL DISTRIBUTION AND PREVALENCE

Hydrophobia is reported as having occurred with more or less intensity in almost all parts of the world. It is frequent in some of the arctic regions, as well as in parts of the tropics. It is well known throughout most of Europe and many parts of Asia. It is seen in Egypt and all about the Mediterranean, but it is said not yet to have been observed in South or Central Africa. Neither has it been reported in Siberia. Australia is said to be free from it because of the rigid carrying out of the six months’ quarantine law in regard to dogs. For the same reason several other islands are also reported free. It is present in the Philippines. England, because of quarantine and periodic muzzling of dogs, is now reported free from the disease. It is common in many parts of the United States.

Kerr and Stimson, in investigating the prevalence of rabies in this country in 1908, found that it had been reported irregularly in all but ten states in the union. In 1911 Stimson stated that it had been reported in all but six states. As the disease is in most sections a non-reportable one, the statistics at present are probably far below the actual numbers. It may be misleading, therefore, to present maps of the incidence of the disease until we have more reliable data.

The number of people bitten, of course, may far exceed the number of animals biting, and the number of animals biting may be greatly exceeded by the number actually affected with rabies. This latter condition is due chiefly to the number of cases of "dumb rabies." Animals suffering from this form of the disease do not usually bite others. But it must not be forgotten that they may occasionally infect through contact with their saliva. Rabies, then, may be quite prevalent without knowledge of the authorities. There is no question but that the specific germ of rabies present in the saliva of rabid animals varies in virulence and in quantity
in different cases. This no doubt helps to account for the variable number of persons developing the disease after exposure when not treated.

In Stimson's report, which does not include all cases, it is stated that 4,625 people in the United States received treatment during 1911, with 98 deaths. The rabid animals were said to be 3,393 in number.

From time to time in the history of rabies severe local epidemics have been reported during which valuable domestic animals have been lost. Then, through extra precautions on the part of the people, the disease has died down again.

**Animals Affected.**—It is essentially a disease of mammals, though all warm-blooded animals under favorable conditions may be infected. Thus it has been reported very infrequently in certain birds. Those animals that are most often subjected to the bites of canines are most frequently affected.

Neither sex nor age is a direct factor in susceptibility; but in humans more children are infected by bites because more come in contact with dogs in their play; and in dogs more frequent cases occur among animals one to four years of age because these, by their greater activity, run more chances of coming in contact with a stray rabid animal.

The dog is the most frequently affected, probably because he wanders more at large. In countries where wolves are common these animals often stand next to the dog in frequency. In well-settled countries cattle seem to be the next most frequently affected, probably because they stand a greater chance of being bitten by wandering mad dogs. Possibly more cats are affected than is known, since cats often develop the paralytic form of the disease, and therefore may not be diagnosed.

The following is an average estimate of number and kind of the animals more usually affected. Of course the percentages vary with the different epidemics and in different countries.

<table>
<thead>
<tr>
<th>Canines</th>
<th>Cattle</th>
<th>Cats</th>
<th>Horses</th>
<th>Goats</th>
<th>Sheep</th>
<th>Pigs</th>
<th>Humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dogs</td>
<td>Wolves</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>85 per cent.</td>
<td>Irreg. in limited areas</td>
<td>8</td>
<td>5</td>
<td>0.1</td>
<td>1</td>
<td>0.5</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Other animals, such as deer, foxes, etc., may be infected irregularly, according to the conditions of exposure.

We know little about the natural infection of the species of animals used in the laboratory for diagnostic tests and for the production of vaccine, such as rabbits, guinea-pigs, rats, and mice. There is an idea in England that rabbits help to propagate the disease in nature. In this coun-
HYDROPHOBIA

try it is thought in some parts of the West that certain skunks are natural carriers. No proof has yet been given of the truth of these ideas.

Another opinion is that rats and mice are capable of perpetuating the disease without the intervention of the dog. But that they play slight, if any, part in the propagation of rabies is shown by the good results obtained in England and other countries where preventive measures are taken against the dog alone.

Birds seem to have a relative immunity, since old fowls and pigeons, if they take the disease, frequently recover. And if they die their nervous system is said to be innocuous to rats, on subdural inoculation, although it may transmit the disease to other fowls. Their relative immunity may be partly helped by their high body temperature (108° F.).

Only a comparatively small percentage of animals bitten by rabid animals develop the disease. The number is closely proportionate to the intensity and site of the bites.

Cases of developed human rabies are now comparatively infrequent owing to the wide application of the preventive treatment. And the earlier data from which the statistics were compiled were very incomplete. However, we may get a general idea of the percentage of incidence in man after bites, before the Pasteur treatment was established, by the following table of Babes, in which the mortality is arranged in order of site and intensity of bites from different animals.

<table>
<thead>
<tr>
<th>Character and Site of Bites</th>
<th>By Wolf</th>
<th>By Cat</th>
<th>By Dog</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple and deep wounds about eye, nose or lips.</td>
<td>100</td>
<td>70</td>
<td>60</td>
</tr>
<tr>
<td>Multiple and deep wounds about other parts of face.</td>
<td>80</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Multiple and deep wounds on other parts of uncovered body</td>
<td>40</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>Single and deep, on finger or neck</td>
<td>20</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>Deep, on well-covered parts of body</td>
<td>15</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>Superficial, on uncovered parts of body</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Same with hemorrhage</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Contact of recent wounds with infected saliva</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Contact of wounds more than 24 hrs. old.</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

This gives a general average from dog bites of 24 per cent., which is rather higher than that given by most authors.

In dogs, after dog bite, the average mortality is about 40 per cent.

Bites of herbivora and of man are very slightly dangerous. There are no authentic cases of transference from man. Glands from humans have seldom been found to be infective for test animals.

Seasonal Prevalence.—The disease is not fundamentally affected by
the time of the year. If more cases are reported during the summer months the larger number is only apparent or accidental. It may be due to the fact that strays are more frequently seen and more easily caught at this time, because more people are abroad. For the same reason more people may be bitten in summer. This applies particularly to the country. In the larger cities the cases of rabies in the dog are often more frequent during the winter.

**PATHOLOGY**

It is not known exactly how the rabies germs act immediately after their introduction into the system. Evidence tends, however, to show that they pass chiefly, if not exclusively, along the nerve fibers probably in the surrounding lymph spaces to the brain. Their occasional presence in the blood is only accidental and transient, as the leukocytes in all probability quickly destroy them. Once within the nerve fiber, they seem gradually to develop. They progress so slowly along its course that they do not disturb its function. When the brain is reached they enter the nerve cells, which they first stimulate and then destroy. This process explains all of the symptoms and the pathologic findings.

**Gross Pathology.**—On autopsy, as might be expected from the action of the organism of rabies just described, no characteristic changes are evident. The fact that no marked changes are found might be considered in itself characteristic.

The central nervous system is often congested. Pin-point hemorrhages and areas of softening may be seen on section. The salivary glands of the dog are also often congested, as are the thyroid, the pancreas, and the suprarenal capsules. Small hemorrhages may also be found in the lungs, and the mucous membranes throughout the body may show catarrhal changes.

The condition of the stomach in the dog has been considered diagnostic, but it cannot be relied upon by itself. This organ frequently has no food particles. It is contracted over a more or less large mixture of foreign substances such as pieces of cloth, hair, leather, wood, and straw. The bile-stained mucous membrane often shows hemorrhagic erosions.

**Histologic Pathology.**—Many histologic studies have been made of the central nervous system. The first abnormal changes that strike the eye on examining under the microscope a stained section of the spinal cord in rabies are groups of small spheroidal cells surrounding many of the blood vessels and the large nerve cells. They are especially marked in the anterior and posterior horns. Similar collections of cells are also seen in the white substance along the connective-tissue septa. These cell collections were early described by many observers. Babes (1892) corroborated these findings, called the groups “rabic tubercles,” and came to
the conclusion after much control study that they were pathognomonic for rabies. But sometimes these groups are not found in cases of rabies; especially are they absent in the early stage; and somewhat similar groupings of spheroidal cells have been found in other forms of disease of the central nervous system. Their use in diagnosis, therefore, is limited.

Many other degenerative changes in the central nervous system were described after this as occurring throughout the course of the disease, but none were found to be absolutely characteristic for rabies. The most important of these changes were found by Van Gehuchten and Nelis (1900) in the cerebrospinal ganglia. In a normal ganglion the large nerve cells are seen lying closely together, inclosed in an endothelial capsule. In a rabies ganglion, on the contrary, many of the large nerve cells have disappeared and their places are taken by groups of small infiltrating spheroidal cells, and by proliferated cells of the capsules.

These changes are found most distinctly and frequently in dogs, least so in man. They may appear quite early; so, while not absolutely specific, may be of help in diagnosis.

Then came the most important histologic discovery of all—that of the specific cell inclusions called Negri bodies. These bodies will be described under Etiology.

A little later Lentz described certain degenerative cells which he found in “passage” animals, that is, animals which are being inoculated successively with rabies virus, beginning with street rabies. Such cells, however, are seen in other conditions, so are not characteristic for rabies.

The whole process in the central nervous system has been classed as an acute parenchymatous encephalomyelitis.

ETIOLOGY

It was early demonstrated that the saliva of rabid dogs usually contained the specific virus of rabies. Then the virus was shown to be present in the salivary glands. Finally its chief site proved to be in the central nervous system. Any part of the brain or spinal cord of an animal dying of hydrophobia inoculated subdurally or intracranially into a susceptible animal always produces hydrophobia in that animal. Not only this, but very small amounts, that is, very high dilutions of the rabies nerve tissue, may cause the disease, though not so regularly. This shows that the virus is present in different animals in different amounts.

Inoculations from dilution emulsions of the saliva or the salivary glands do not so uniformly produce hydrophobia. This shows that the sputum does not always contain as many organisms as the brain. The virus is practically always present in the submaxillary glands of dogs, but is not always found in the parotid or the sublingual glands.
In herbivora the glands, and consequently the sputum, are still less regularly virulent. In man they are probably least virulent of all.

This rabies virus, found so abundantly in the nerve substance, has been the subject of innumerable studies. It constitutes a pure culture of the rabies organism; and, though the question of artificial cultivation is unsettled, we have learned many facts which are of practical importance in its application to the vaccine treatment of man.

Cultivation of Parasite of Rabies.—Noguchi reports the successful cultivation of the organism producing rabies (hydrophobia). He describes the organisms grown in the cultures as very minute granular and somewhat coarser bodies, some of which resemble Negri bodies, and Noguchi states that they can be transplanted in new cultures through many generations. He says he has reproduced rabies in dogs, rabbits, and guinea-pigs inoculated with these cultures.

Williams has raised the question as to whether or not Noguchi has actually grown the parasite of this disease and has not carried over in his cultures some of the original material. Consequently animals inoculated with this material would come down with hydrophobia, irrespective of any growth of the organism outside of the body. The larger bodies described by Negri are no doubt nonspecific crystalline substances. They have been found by us in tuberculous sputum as well as in ascitic fluid. Noguchi's work has not yet been corroborated.

Response of Rabies Virus to the Action of Physical and Chemical Agents.—That this virus is more resistant to certain agents than artificial cultures of many known organisms is thought to be due partly to the fact that it is surrounded by the brain substance which might hinder the action of the agents employed. Poor and Steinhart have considered this in their studies on "Rabies Virus Freed from the Cells of the Host." They obtain the virus from the glands by aspiration, filter it through a Berkefeld filter, and study the action of certain agents on this comparatively freed virus. They come to the conclusion that the two viruses (brain and gland) are similar in their reactions to the agents tried.

The fact that the virus resists the action of glycerin for so long a time has a practical bearing in certain methods of treatment. It is of use also in ridding decomposed brains sent in for diagnosis from contaminating bacteria before making the animal inoculations.

The degree of resistance of rabies brains and spinal cords to different methods of drying and heating has also been made use of in preparing vaccine for treatment. Thus slow drying at a moderate constant temperature (about 20° C.) causes a gradual loss of virulence (see Classic Pasteur Treatment). Very rapid drying at any temperature up to 36° C. preserves much of the virulence (see Harris’ method of treatment, 727). Under exclusion of air and in a moist condition in the dark the virus preserves its virulence for 2 months at 23° C., and for 22 days
HYDROPHOBIA

at 35° C. It is killed, however, in 4 hours at 45° C., in 20 minutes at 50° C., and in 5 minutes at 60° C.

When kept in a cool, dark place, protected from the air, the virus remains virulent for a long time in brains which have become contaminated with many organisms. Thus the decomposed brains of rabid animals may produce rabies on inoculation into test animals after being buried for many months. They must, however, be rid of the contaminating bacteria first, either through filtration or by the prolonged action of glycerin.

The action of certain chemical disinfectants on an emulsion (1 to 100 normal salt solution) of fresh rabic brains may be shown by the following table:

<table>
<thead>
<tr>
<th>Agent</th>
<th>Strength, per Cent.</th>
<th>Time of Exposure, Hours</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbolic acid</td>
<td>1</td>
<td>24</td>
<td>Non-virulent</td>
</tr>
<tr>
<td>Carbolic acid</td>
<td>5</td>
<td>20</td>
<td>Non-virulent</td>
</tr>
<tr>
<td>Mercureic chlorid</td>
<td>1</td>
<td>24</td>
<td>Non-virulent</td>
</tr>
<tr>
<td>Formaldehyde (Cumming)</td>
<td>0.08</td>
<td>2</td>
<td>Non-virulent</td>
</tr>
</tbody>
</table>

The best disinfectant for contaminated places and fabrics is boiling water when possible, otherwise formalin.

Filterability of the Virus.—It has been known since 1903 (Remlinger) that rabies virus, under certain conditions of dilution and suction, passes in part through a Berkefeld filter. Street virus from the central nervous system passes less readily than fixed virus; but gland virus from street cases (dogs) passes most readily of all (Poor and Steinhart).

Negri Bodies

From the earliest days of the etiological studies of rabies many formed elements have been described as the looked-for specific organisms. But each lacked confirmation until Negri and others demonstrated that the structured cell inclusions now known as Negri bodies are probably the specific micro-organisms causing rabies. They have been made the subject of extensive studies by many investigators, among them Williams and Lowden in 1906. As a result of a series of studies on the nature of these bodies Williams concluded with Negri that they are probably protozoa and the cause of rabies. Williams gave them the name Neuronyctes hydrophobie, and presented as her reasons for considering them micro-organisms the following facts:
ETIOLOGY

1. The bodies show distinct characteristics in both morphology and stain.

2. Their morphology is constantly cyclic, i.e., a definite series of forms indicating growth and multiplication can be demonstrated: small, single, rounded or oval plastin-staining granules; similar forms in twos or groups; larger forms containing a definite central or eccentric chromatin mass (nucleus); forms with smaller chromatin masses arranged in a ring about the central mass; evidence of division of these larger forms as well as of the small ones; segmentation of chromatin and distribution of nuclear-staining material throughout the whole organism; division of the organisms into many minute bodies; and finally, from the beginning of the appearance of the smaller masses of chromatin, all stages of budding, a phenomenon which accounts for the appearance in the same cell and at the same time of both large and small forms, and also helps to account for the rapid spread of the organism and for forms small enough to pass certain filters.

3. In rabbit "fixed virus," besides the few larger forms seen by others, very many extremely minute forms are found, within most of which are seen, in well-fixed and stained preparations, a single chromatin granule.

4. With stains such as Giemsa's the lightly basophilic property of the "cytoplasm" of the bodies and the chromatin-like nature of the contained masses and granules are well brought out, better in spreads than in sections.

5. Negri bodies are found in all parts of the infectious central nervous system, beginning to appear in the large nerve cells as extremely minute forms before the beginning of symptoms, i.e., on the fourth day in rabbit fixed virus infections and on the seventh day in rabbits inoculated with street virus; thus they are found early enough to account for the infectivity of the host tissue.

Most of these findings have been confirmed. Recently Watson states that he has found in addition spore-like bodies similar to myxosporidian spores.

Others have brought forward other explanations of the nature of these bodies. All of these hypotheses may be summarized as follows:

1. Negri bodies are micro-organisms and the cause of rabies (Negri, Williams and Lowden, and many others).

2. The plastin-staining portion of the bodies is due to the host cell reaction to the specific micro-organisms, which are the small chromatin masses seen within the bodies (Volpino, Babes, Prowazek, and many others).

3. They are due to the cell reaction to the rabies toxin. They may or may not contain the organisms (Marie and others).

4. They are extruded and degenerated nucleolar material (Acton and Harvey).

Something may be said in favor of each of these hypotheses except the fourth; but the balance of evidence seems to us still to be greatly on the side of the first one.

Whatever the nature of these bodies, their practical specificity has been accepted, with the result that all over the world their presence is considered proof that the disease is hydrophobia. The methods for the practical demonstration of these bodies are given under the heading Diagnosis.
INCUBATION

The fact that the incubation period in this disease is usually very long is one of chief importance in the treatment of the disease by prophylactic vaccine. It gives time for the long series of inoculations which have been considered the best way to produce immunity in the cases bitten.

The time from the bite to the appearance of the first visible symptoms varies usually according to the number, severity, and site of the bites. Rarely is it earlier than 12 days or later than 90. The limits given are 8 days and several years. In most cases of humans it occurs in from 3 to 8 weeks. The statement of any time over a year must be received with caution. Few cases beyond that time have absolutely reliable data. It is seldom that all sources of possible intercurrent infection, such as contact with the saliva of another animal before it has shown symptoms, can be absolutely ruled out.

The period of incubation varies somewhat (1) with species, being longest in man; (2) with age, being shorter in younger cases, though this may be due to the fact that the younger ones receive more bites; (3) with the site of wounds—the shorter the nerve trunk the shorter the incubation; (4) with the severity of the wounds, in direct proportion; (5) anything that weakens the body, especially the nervous tissue, such as shock, alcohol, syphilis, meningitis, shortens incubation; (6) with the virulence of the strain of infecting virus.

Stimson in 1912 gives the period of incubation in 65 of the cases in man which occurred during 1911 in the following table:

<table>
<thead>
<tr>
<th>Days</th>
<th>10 to 20</th>
<th>21 to 30</th>
<th>31 to 40</th>
<th>41 to 60</th>
<th>61 to 120</th>
<th>121 to 240</th>
<th>241 to 365</th>
<th>Over a year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>11</td>
<td>19</td>
<td>9</td>
<td>8</td>
<td>10</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

Average, 49.25 days.

For lower animals the following table may be accepted:

<table>
<thead>
<tr>
<th>Animals</th>
<th>Average</th>
<th>Longest</th>
<th>Shortest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog</td>
<td>2 to 8 weeks</td>
<td>1 year</td>
<td>8 days</td>
</tr>
<tr>
<td>Cat</td>
<td>2 to 4 weeks</td>
<td>1 year</td>
<td>7 days</td>
</tr>
<tr>
<td>Cattle</td>
<td>1 to 3 months</td>
<td>3 years</td>
<td>2 weeks</td>
</tr>
<tr>
<td>Horse</td>
<td>2 to 8 weeks</td>
<td>20 months</td>
<td></td>
</tr>
<tr>
<td>Swine</td>
<td>2 to 4 weeks</td>
<td>6 months</td>
<td>6 days</td>
</tr>
</tbody>
</table>
SYMPTOMS

The symptoms in all animals present the same general characteristics and point plainly to the cerebrospinal system as the site of the chief lesions. They may be divided into two groups or stages: (1) those of excitation or irritation; (2) those of degeneration. If symptoms due to excitation predominate the disease is called furious rabies or hydrophobia, if symptoms of paralysis quickly appear, due to rapid degeneration of the nerve centers, the disease is called dumb or paralytic rabies or hydrophobia. Many cases present a mixed type of symptoms, and a few are quite atypical.

Some details of the manifestations of the disease as it occurs in the most common biting animal (the dog) may be given in order to help us determine whether or not, in any case, the biting dog is mad and the bitten one needs treatment.

Symptoms in the Dog.—Type I, Furious Rabies.—Stage of Excitation.—There may be a slight fever before symptoms are apparent. The first thing noted may be a change of character. A non-affectionate dog may become affectionate and an affectionate one non-affectionate. The increased affection may show itself in more frequent attempts to lick the hands and face of the owner. Even at this time the sputum may be virulent, so we should beware of unnatural playfulness, especially if we know the dog has been bitten. The appetite is variable and may already be abnormal. The unnatural activity and restlessness may gradually become more marked during two to four days. The animal may appear to have hallucinations, such as seeing imaginary flies and fearing inoffensive objects. Later he loses fear and begins to bite at things and animals, especially at dogs, lastly at man. He may run far away from home in this stage; in fact this is often the first symptom noted by unobservant owners. It is during such runs that he bites people and other animals. He may return exhausted and be quieter for some time. He may even seem normal again; he may recognize his master, respond to caresses, and eat and sleep. Then he again becomes excited. If in a cage he moves constantly. In a room he may bite and tear things. His bark early becomes characteristically altered in pitch and mode. It is described as changing from a succession of resonant sharp barks to a low howl followed by an irregular series of low pitched barks between uninclosed jaws. Some dogs do not bark characteristically; some do not bark more than usual. The majority do not have fear of water. They drink as long as they can; that is, until local and general convulsions are marked. The saliva drops, but the animal may not “froth at the mouth.”

Stage of Paralysis.—The first stage passes insensibly into the second. Signs of weakness of certain muscles appear, often in the posterior extremities, sometimes in the anterior, and sometimes in the muscles of the jaw or other muscles of the body. The local and general tonic-clonic spasms gradually become less. The pupils are dilated. Respirations and heart beat are irregularly increased. Paralyses increase and death finally supervenes. The duration of obvious symptoms is usually 4 to 6 days, not infrequently 7 to 8 days, practically never over 10 days. The stage of irritation is from 3 to 4 days; the stage of paralysis is from one to two days.

Dumb Rabies.—Fifteen to 20 per cent. of cases among dogs occur in this form in nature (Högyes). The stage of excitation may be so short as to be unnoticed. Paralysis may be the first symptom observed, often first in the lower jaw, causing
"drop jaw," which makes the owner think that his dog has "a bone in its throat." The animal does not bite anyone when suffering from this type of the disease, but its sputum may be as virulent as that of a biting animal, and may cause rabies if it comes in contact with any recent wound, even that produced by a "hang nail." The posterior extremities and the rest of the body quickly become paralyzed. Paralytic rabies is said to be a more intense form of the disease, but that this is not so in certain cases seems evident both from the clinical history and from the amount of infective material found in the central nerve system and in the salivary glands. We have had several cases of "drop jaw" which have lasted a longer time than the other forms of the disease.

Poor says that he has seen a few cases of drop jaw of uncertain origin which have become well. Whether or not these are spontaneously cured cases of rabies we cannot say.

**Symptoms in Man.**—The psychic and reflex symptoms, which are usually the first to appear, are similar to those following any excitant, causing hypersensitiveness of the nerve cells, therefore they may be easily simulated. But one is not left long in doubt. Sometimes the first symptom is a local irritation of the wound, a tingling and itching, accompanied by some engorgement and pain. This may be simulated by neuralgic pains from any cause. Sometimes the patient complains of a sensation of constriction in the throat, or of difficulty in walking or breathing, or of precordial anxiety, or of neuralgic pains in other parts of the body. There is usually a moderate rise of temperature (38° to 39° C.). These indefinite symptoms generally last about 48 hours. During this time one of the most characteristic symptoms in man, the fear of water, may develop. It is not always marked. It is simply due to the painful spasm of the muscles of deglutition from the attempt to swallow. Of course the more the patient feels he cannot drink the more thirsty he becomes, hence the reason why the greatest fear seems to be of water. Solid foods are more readily taken than fluid. There is a characteristic pharyngeal and respiratory spasm on exposure to a draft of air (aërophobia). Loud speaking may also cause spasms (hyperacusis). Remissions, which may give the friends short hope of recovery, usually occur, except in the very severely infected cases. At about 48 hours the periods of excitement become more marked. There may be hallucinations, and even mania; but there is seldom a tendency to injure others, even by biting. The patient realizes what he has done between the attacks. Indeed, his mind is irregularly clear until near the end. His voice becomes hoarse with a peculiar quality. There is never any real barking, though sometimes the noise the patient makes slightly simulates it. The eye symptoms are photophobia, nyctagmus, and sometimes strabismus. The pupils are unequally dilated.

Vomiting is frequent, and may be dark-colored as a result of hemorrhage or regurgitated bile, or both.

Death may occur after 1 to 4 days during this stage suddenly through
apoplexy or asphyxia, or after a short period of apparent agony. But usually the patient passes into the paralytic stage.

Paralytic Stage.—The muscles relax, the jaw drops, ropy saliva flows, the face becomes smooth and expressionless, the patient becomes comatose, breathing becomes irregular and feeble and finally stops. The temperature increases just before death; it may be as high as 42° C. [The Editor has known a temperature of 44.5° C. (112° F.) to occur in man an hour before death.—B.] The pulse is generally irregular and over 100. Just before death the blood shows a leukocytosis. Sugar and acetone may be found in the urine, but no albumin. This stage lasts from 2 to 18 hours.

Paralytic Hydrophobia (Dumb Rabies).—This type of the disease, which includes those cases that show almost from the beginning symptoms pointing to degeneration of the nerve tissue, is less recognized in man than the former type, and probably has been sometimes incorrectly diagnosed.

From the standpoint of treatment its recognition is necessary in order to be able to differentiate it from the paralyses which occur occasionally during or just after the Pasteur treatment.

Though this form of the disease was described long before the Pasteur treatment came into use, it had been forgotten, and when cases occurred after the treatment many people said that Pasteur gave the disease instead of curing it. The proof of the relation of the disease to street rabies, rather than to laboratory rabies, in these cases is given by animal inoculations.

The onset in humans is the same as that of the convulsive type, but shorter. Then the lower extremities feel heavy and numb. They become quickly ataxic and then paralyzed. The paralyses spread irregularly. Death occurs usually from heart paralysis in from 2 to 8 days. Consciousness is retained until late in the disease.

**DIAGNOSIS**

In man rabies must be differentiated from hysteria or lyssophobia, from delirium tremens, from tetanus, and from the action of several poisonous drugs.

The history of the case must be determined if possible. A negative history with the absence of reflex irritation to stimuli, especially to air, will usually eliminate rabies.

The following points should be considered in history-taking: (1) exposure to infection from the biting animal and of this animal; (2) length of incubation in each; (3) symptoms; (4) termination; (5) post-mortem finding; (6) incubation tests of animals.
HYDROPHOBIA

In hysteria or lyssophobia the reflex response to stimuli is never so intense as in rabies, and the symptoms are amenable to suggestion.

In tetanus the spasms are tonic, with continued contraction of the jaws instead of alternate relaxation.

In the dog a positive clinical diagnosis can usually be made by an experienced doctor if the animal can be under prolonged observation, but the animal so frequently is killed on sight that this sure diagnosis can rarely be given. So we must usually rely upon the laboratory tests.

Since the discovery and proved specificity of the typical Negri bodies their demonstration has been considered positive evidence of rabies.

Williams, in 1904, recommended a rapid spread method for demonstrating these bodies which allows a diagnosis to be made in a few minutes. This method was later perfected by Williams and Lowden Van Gieson, Frothingham, Harris, and others, and is now made use of for rapid diagnosis in most parts of the world. We have used the spread method for showing the presence of these bodies since 1904, and we still find it eminently satisfactory. We can still state that we have never obtained negative results from inoculating animals with material that shows typically structured Negri bodies. We must say, however, that we continue infrequently to receive brains that do not show these typical bodies, yet on animal inoculation produce rabies. All of these brains, it is true, show suspicious small forms, and a few of them if kept at 12° to 18° C. overnight show typical bodies the next day. So the percentage of failures by the spread method is very small. Of course decomposing brains which unfortunately are sent for diagnosis quite frequently in the summer-time, cannot always be diagnosed successfully by this method. In these cases, and in the few suspicious cases, animal inoculations must still be made as the final test of the presence of rabies.

In certain cases killed too early in the disease to give time for the general development of the organisms in the brain the salivary glands may be virulent, and since the gland virus in general brings guinea-pigs down with rabies sooner than the brain virus (Poor), the virus from the glands should be inoculated in these cases. It has been repeatedly shown that the sputum may be virulent many days before the appearance of brain symptoms. The longest time reported is 15 days. In such cases, of course, contact with another rabid animal must be absolutely excluded.

The following is the routine method in the New York City Health Department of handling the material sent in for diagnosis:

1. If the material is fresh, spreads are made by pressing between a glass slide and a coverglass a small, thin section of the gray matter from each of the following parts of the brain: (a) the cerebral cortex, (b) Ammon's horn, (c) the cerebellum. The material is spread along the slide by moving the coverglass down with the finger. Experience teaches the amount of pressure to be used.

2. When partly or completely air-dried the smears are fixed for about ten
TREATMENT

seconds in neutralized \(^1\) methyl alcohol (C. P.) to which one-tenth per cent. of picric acid has been added. The excess of the fixative is removed by blotting with fine filter paper.

3. The fixed smears are stained in the following solution:

- Saturated alcoholic solution of fuchsin ............... 0.5 part
- Saturated alcoholic solution of methylene blue .......... 10.0 parts
- Distilled water ........................................ 30.0 parts

This solution, which is a modification of the one proposed by Van Gieson for staining the Negri bodies in smears, changes rather quickly at room temperature, but, kept in the icebox, it gives good results for an indefinite time. The stain is poured on the smear and held over the flame until it steams. The smear is then washed in tap water and blotted with fine filter paper.

With this stain the Negri bodies appear a magenta color, the nerve cells blue, and the red blood cells yellow or salmon color.

Giemsa's stain gives brilliant results, but it requires more time than the above stain, and therefore is not as good for diagnostic work. The other methods published of demonstrating these bodies we have found to possess no advantage over the one given here.

4. If nothing is found smears are made from various other parts of the brain.

5. If still nothing is found an emulsion is made in 10 c. c. normal salt solution of a piece the size of a bean from the different parts of the brain, and an intracerebral inoculation (\( \frac{1}{4} \) c. c.) is made into each of three guinea-pigs.

6. Pieces of the brain are also put into sterilized neutral glycerin for later inoculations, if for any reason the first should fail. Brains so preserved remain active in the icebox for over three months. By this method contaminated brains lose many of their extraneous organisms.

7. An emulsion made from the contaminated material preserved in glycerin is inoculated after two weeks, unless positive results have been gotten from the first inoculation.

8. Sections may be made, if the brain is very soft, but usually this is not necessary.

TREATMENT

The treatment of hydrophobia is essentially one of prophylaxis by means of a specific vaccine. Until recently serum has played a very small part in the treatment, and drugs none at all, except in alleviating the symptoms of the developed disease.

The hope of finding a curative serum has not been fulfilled, and we still have no known specific cure for rabies after the process has become established in the central nervous system. If recovery occurs in man after the appearance of symptoms it is extremely rare. A few cases of cure after this stage have been reported, but no proof that the diagnosis was correct has been given. In the disease as it occurs in nature the question is difficult to settle, since slight symptoms, especially in the

\(^1\) The wood alcohol is neutralized by adding sodium carbonate, about .25 gms. to 500 c. c. of the alcohol.
lower animals, may be overlooked, and in humans may be attributed to nervousness or other causes. It has been proved, however, that experimental animals occasionally may show symptoms and recover spontaneously, therefore such a possibility cannot be ruled out in man; and one may still hope for a cure at a later stage, if not from a specific serum, perhaps from a special drug.

**Prophylactic Treatment.**—The prophylactic treatment may be considered under two heads, local and constitutional.

**Local Treatment.**—The wound should be treated as any infected wound. Free bleeding may be encouraged, and immediately the wound should be cleaned with any fluid antiseptic solution, and should then be cauterized with fuming nitric acid. Babes suggests bathing the wound with antirabic serum.

**Constitutional Treatment.**—This consists chiefly in the use of the specific vaccine. If we could determine easily in each case the presence of rabies in the source of infection the procedures as to treatment would be comparatively clear cut, but unfortunately there are several factors which interfere with an immediate and definite diagnosis: (1) The biting animal may be a stray who has disappeared after the biting; (2) he may be an apparently healthy animal; (3) he may have indefinite symptoms; (4) he may be killed before clinical or microscopic manifestations appear; (5) he may be sent to the laboratory without a history and in too bad a condition for microscopic diagnosis.

If we cannot rule out these factors we cannot rule out rabies, and unless we can rule out rabies we should be guarded in advising no treatment, especially in communities where cases of rabies have been reported.

The antirabic treatment should be advised, therefore, when any of the following conditions obtain:

1. When the animal shows clinical or microscopic signs of rabies.
2. When the animal has disappeared just after biting and cannot be found. This in itself is a suspicious symptom, especially if the animal is a stray. All efforts, however, should be made to obtain further facts in regard to the appearance and actions of the animal. The apparent reason for the bite and the successive biting of other animals or people must also be borne in mind, as well as the fact that the bitten person’s imagination may be colored by the wrong ideas of the disease that are so common among the laity.
3. If the biting animal has been killed before it can show microscopic evidence of rabies, or if its brain is sent to the laboratory in too bad a condition for immediate diagnosis, and if the bite is severe and unprovoked, beginning treatment should be advised pending the results of inoculating test animals. If these test animals show no symptoms in 14 days the treatment may be stopped if one of the longer methods is being used, to be begun again if the animals show symptoms later.
(4) If an apparently healthy animal or one with only slightly suspicious symptoms should bite a person, the advice should be to keep the animal under observation for at least two weeks, and to begin treatment if suspicious symptoms appear or become more marked. This is the longest period between the biting and the appearance of symptoms that a dog's sputum has been shown to be virulent.

(5) If the animal is killed and no evidence can be gotten for or against rabies, treatment should be advised in areas where rabies is prevalent.

(6) When cases have been only exposed to an animal’s saliva, unless we can absolutely rule out fresh cuts or abrasions on the parts exposed, treatment should be advised.

Probably all of those cases cited where rabies is said to have developed without bite or contact are in reality contact cases. The slightest fresh abrasion from whatever source may be more dangerous than deeper clean-cut wounds which bleed freely, but they may be more easily overlooked.

**Specific Treatment**

The specific or antirabic treatment of hydrophobia in man dates from 1885, one year after Pasteur had made his first announcement to the French Academy of the results of his extensive studies on this disease. In this year Pasteur, with Roux and Chamberland, gave the results of further experiments on methods of obtaining a less virulent virus for use in beginning protective inoculations. The method which they recommend became known as the Pasteur Method, and with certain modifications it has been and still is used all over the world.

"**Street Virus**" and "**Fixed Virus**."—In Pasteur's first attempts to immunize against rabies certain difficulties were encountered, chief among which were: (1) the inability to obtain an invariable inoculation dose, owing to the irregular strength of the virus as it occurred in nature, or in “street rabies,” as it was called; (2) the inability to obtain a surely attenuated virus that would produce immunity and still be harmless.

To overcome the first difficulty, i. e., to get a virus that would always produce rabies in essentially the same dose, Pasteur tried passing the virus successively through different species of animals by subdural inoculations. He found by this method that in certain animal species (e. g., monkeys) street virus became attenuated, while in others (notably rabbits) it became markedly increased in virulence, as evinced by the shortened incubation period. The attenuation in virulence by passing through less susceptible animals occurred much less regularly than the increase in virulence by passage through more susceptible species, therefore he discarded the passage method of attenuating the virus, but continued working to increase the strength. He
found that after many passages through rabbits (about 50) the virus present in the central nervous system (the medulla was mostly used) of the rabbits dying from the infection would, when inoculated into a fresh rabbit, bring it down with the disease in a fixed time. He called this virus *virus fixe* (fixed virus), and used it as the basis for further operations in preparing his vaccine treatment. He found a way to attenuate this virus more or less regularly and so to obtain gradually increasing doses up to the fully virulent virus. He also found that this rabbit-fixed virus did not so often cause death in animals when given subcutaneously as did street virus. Later others found that by inoculating themselves subcutaneously with emulsions of fully virulent fixed virus no ill effects were produced, at least in some human beings, by this method of inoculation.

Högyes states that he obtains a fixed virus sooner (in 16 passages) if he uses only young rabbits. Babes claims that many strains of street virus will become fixed for the rabbit by three to four preliminary passages through the guinea-pig.

The strain of fixed virus in one institute may differ slightly in strength from that in another.

Babes gives the following table of loss of virulence by drying in fixed virus cords of different institutes:

<table>
<thead>
<tr>
<th>Location</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paris and St. Petersburg</td>
<td>5-6</td>
</tr>
<tr>
<td>Bucharest</td>
<td>4</td>
</tr>
<tr>
<td>New York, Moscow</td>
<td>6</td>
</tr>
<tr>
<td>Kiev, Sartou</td>
<td>6-7</td>
</tr>
<tr>
<td>Tomsk</td>
<td>9</td>
</tr>
</tbody>
</table>

**Inoculation of Rabbits for the Production of Fixed Virus.**

The animals may be etherized, but, if the skin over the point of the cut be washed just before the operation with 5 per cent. carbolic solution, the very slight pain from the skin cut necessary is deadened by the anesthetic action of the carbolic acid. The rest of the operation produces no pain. A fourth of an inch incision through the skin is made back of the eye on one side of the median line, the skin is held apart and a small opening is made with a stylet through the skull bone just large enough to admit the fine short (1/4 inch) hypodermic needle of the syringe containing the emulsion (0.2 c. c.) to be inoculated (Fig. 1). The inoculation is made intracerebrally. When the needle is withdrawn the skin is allowed to come together, and, though no further treatment of the wound is necessary, it is usually covered with a little cotton and collodion.

**The Classic Pasteur Treatment.**—The various steps in the classic Pasteur treatment may be summarized briefly as follows: (1) obtaining a fixed virus by successive passages of street virus through a rabbit; (2) removing aseptically the spinal cords of rabbits dying from such fixed virus infection and drying the cords over caustic potash at 20° C. (70°
TREATMENT

F.) in order to attenuate the virus until no virulence is shown in test animals (from eighth to tenth day, according to Pasteur); (3) inoculating patients subcutaneously on successive days with emulsions from measured quantities of these cords, beginning with the dried avirulent cords and passing to the infective ones until fully virulent material is given.

Pasteur began his inoculations with a 14-day cord, and carried the treatment on for 18 days in the lighter cases and 21 days in the more severe ones. His schemata are given on page 731.

Pasteur's method in its entirety was soon adopted in many lands, and

Fig. 1.—Inoculation of Rabbit for Production of "Fixed Virus."

his results were corroborated. Before long, however, a number of modifications were suggested by different observers, some slight, others more fundamental. Some have been widely used, such as Högyes' dilution method; others have had a limited application in lower animals and are probably only of theoretic interest as regards man. Such are the intravenous inoculation of brain emulsions from street rabies into herbivora (Nocard and Roux, Protopopoff), and the intraperitoneal inoculations of large doses of fully virulent fixed virus into dogs, cats, or rabbits (Helmann, Heim, Remlinger). Immunity has been produced also in rats by allowing them to feed on rabid brains (Fermi, Repetto, Remlinger). Negative results have been reported in other animals by this method.

Högyes in Budapest was one of the first to use a different procedure. He claimed that the virus by Pasteur's method was attenuated only through the death of some of the specific organisms, that is, that there were simply fewer living organisms in the early doses given than in the later and that therefore the same result might be obtained perhaps with even more accurate dosage by giving gradually decreasing dilutions of a
fresh virulent cord. By diluting sufficiently he obtained a mixture which when inoculated did not produce rabies in the test animals, a result similar to that following an 8- to 10-day dried cord. This dilution he used for the first inoculation and gradually stronger dilutions for the succeeding ones. In this way Högyes says he has produced immunity in dogs even from intracranial infection.

Some question the similarity of the two methods. They claim that by the former method the dead bodies or other toxins of the rabies germs contained in the dried cords are able to produce a certain degree of immunity. Poor in our laboratory produced immunity by the inoculation of 9-, 10-, and 11-day cords. But such cords probably always contain a few living germs—not enough, however, to cause death.

Other methods of attenuating or diluting fixed virus have been used, such as exposure to the action of heat, cold, gastric juice, glycerin, or carbolic acid.
The mixed treatment with specific serum and vaccine has also been employed chiefly by Marie, by Remlinger, and by Babes.

Some details of the more important of these methods with the number of cases treated by them, the percentage mortality, and the complications may be considered. In making up statistics for mortality from rabies we must always consider the time allowed for the establishment of immunity by the treatment. This has been found to take place in about 15 days after the last inoculation. Any deaths that occur within 15 days after treatment is finished are considered to have been too severely infected or too susceptible or infected with too virulent a virus to have given time for the production of immunity by the treatment. Therefore, two figures should always be given in mortality statistics: the absolute mortality and that occurring beyond the 15-day limit. Dr. D. W. Poor has
kindly given valuable assistance in preparing the following descriptions.

Method of Attenuation by Gradual Drying.—The method of drying the cords slowly at a moderate heat is the classic method of Pasteur. It has undergone modifications in three general directions: (1) lengthening or shortening the period of treatment; (2) starting the inoculations with a less attenuated cord; (3) increasing or decreasing the amount given at each injection. The method of drying the cords, however, has remained essentially the same as that used by Pasteur.

Fig. 4.—One corner of constant temperature room showing drying bottle containing fixed virus cords being prepared for vaccine.

The cord is removed by a modification of the method of Oshida in the following manner: Strict asepsis is preserved. The rabbit when completely paralyzed (seventh day) is killed by gas or chloroform and is dropped into a 5 per cent. solution of carbolic acid for 5 minutes. It is then removed, the excess of carbolic solution is drained off, and an incision through the skin at the upper and inner part of the thigh is made. The skin is loosened by cutting around the lower portion of the trunk. It is then pulled by the hands toward the upper extremity of the animal and over the head to the ears, leaving the back exposed and sterile throughout the entire length of the spine (Fig. 2). The spine is then divided transversely near each extremity by bone-cutting forceps. The muscles are cut through about these areas so the spine may be more easily reached. With a long wire probe swabbed with cotton at one end the cord is pushed
upward from its canal, freed from its nerves and membranes. The spine is steadied by lion-jawed forceps. The cord curls in a spiral as it emerges and rests on the sterile muscles of the neck (Fig. 3). It is lifted with forceps placed in a Petri dish and cut in two. A small piece is cut from one end and is dropped into a tube of broth to test its purity. A ligature with one long end is placed about each piece, both of which are then hung in a drying bottle (Fig. 4).

Drying the Cord.—The drying bottles are sterile aspiration bottles with both openings plugged with cotton. A layer one inch high of sticks of caustic potash covers the bottom, and the pieces of cord are suspended from the top cotton plug by their attached ligatures. The bottles are then labeled and placed in the constant temperature room (Fig. 4) or incubator, which is kept at a temperature of about 21°C. (70°F.). After 24 hours' drying the cord is known as 1-day cord, after two days, as 2-day cord, etc. Pieces of cord cut off at any time and put into glycerin will retain about the same strength for several weeks. This procedure is followed in regions where there are few cases of rabies, and the daily killing of rabbits to keep up the vaccine would be a large expense. It may also be followed where treatment is sent by mail.

The schemata devised by Pasteur which have been most used may be tabulated as follows:

<table>
<thead>
<tr>
<th>Days</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
<th>17</th>
<th>18</th>
<th>19</th>
<th>20</th>
<th>21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light cases</td>
<td>Age of Cords</td>
<td>14</td>
<td>12</td>
<td>10</td>
<td>8</td>
<td>6</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Amount c. c.</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe cases</td>
<td>Age of Cords</td>
<td>14</td>
<td>12</td>
<td>9</td>
<td>6</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Amount c. c.</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The New York City Health Department used the same schemata with slight modifications up to January, 1906, when they began treatment with a 10- and 9-day cord and finished with a 2-day. They continued with this until August, 1913. Since then they have been using one of the more intensive methods first adopted by the Berlin Institute. From 1906 to 1914 inclusive they treated 5,134 cases infected by rabid animals, with a total mortality of 0.46 per cent. and a corrected mortality of 0.18 per cent. They have had 7 cases of definite paralysis with two deaths. About
nine thousand cases in all, including those not bitten by rabid animals, were treated.

Since it had been found that fresh rabbit fixed virus inoculated subcutaneously into man is probably harmless, the Berlin Institute, with the hope of obtaining an earlier immunization and a shorter treatment, began to give still earlier cords. In 1901 it began with the 8th-day cord on the first inoculation, and was inoculating a 2-day cord on the 8th day of treatment. Its treatment lasted 21 days. This method was adopted at the Hygienic Laboratory in Washington in 1908, with slight variations for the different degrees of bites.

Modifications used by the New York City Health Dept. (Fielder):

<table>
<thead>
<tr>
<th>Cord Dried</th>
<th>Scheme 1</th>
<th>Scheme 2</th>
<th>Scheme 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Injections in 21 Days</td>
<td>Number of Injections in 21 Days</td>
<td>Number of Injections in 21 Days</td>
<td></td>
</tr>
<tr>
<td>Ten days</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nine days</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Eight days</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Seven days</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Six days</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Five days</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Four days</td>
<td>5</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Three days</td>
<td>5</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Two days</td>
<td>5</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Number of patients treated</td>
<td>1,408</td>
<td>811</td>
<td>911</td>
</tr>
<tr>
<td>Cases of paralysis</td>
<td>1</td>
<td>6 (one fatal)</td>
<td>0</td>
</tr>
<tr>
<td>Cases of rabies</td>
<td>4</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

Thus it will be seen that Scheme 2 is somewhat stronger than Scheme 1, since it contains three more injections of two-day cord, and much stronger than Scheme 3, which includes nothing more active than three-day cords. Scheme 2 is very similar to the one employed by the Hygienic Laboratory of the United States Public Health Service, Washington, D. C., and by most of those who produce antirabic vaccine in this country, with these differences: 1. The amount of cord per dose was only two-thirds of that employed in the Washington scheme. 2. We used a two-day cord instead of one-day cord on the eighth and twenty-first days of treatment.

**Treatment by Mail.**—The New York City Health Department was the first to send out treatment by mail to physicians for their own patients. Full directions are sent in the mailing case. One-fourth per cent. of carbolic acid is added as a preservative to the emulsions prepared as above for all treatments. The Washington Hygienic Laboratory soon began sending treatment by mail, and recently manufacturing firms have followed suit. The results from the treatment sent in this way seem to be equally as good as those from the treatment administered at the laboratory.
**TREATMENT**

**More Intensive Treatment.**—In Berlin, where intensive treatment has been longest used, they began to employ even fresher cords for beginning doses because they continued to have late deaths, though not quite so often, after the more intensive methods they were using. Since 1910 Joseph Koch, the present chief of the Institute, has been using the following schema:

<table>
<thead>
<tr>
<th>Days</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
<th>17</th>
<th>18</th>
<th>19</th>
<th>20</th>
<th>21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of Cords</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

The dose is 2 c.c. of cord emulsion (1 part of cord in 5 parts of sterile physiologic salt solution) inoculated once a day into the subcutaneous tissue of the abdomen. Children and adults receive the same dose.

Simon gives the following statistics of the results of Berlin's increasingly intensive methods:

**Berlin Statistics**

<table>
<thead>
<tr>
<th>Period</th>
<th>Age of cord used for beginning inoculation</th>
<th>Cases</th>
<th>Paralyses</th>
<th>Mortality</th>
<th>Per Cent.</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. 1898–1906</td>
<td>Chiefly 8-day cord</td>
<td>2,896</td>
<td>0</td>
<td>21</td>
<td>0.7</td>
</tr>
<tr>
<td>II. 1906–1909</td>
<td>4-day cord. Sometimes</td>
<td>1,490</td>
<td>2</td>
<td>7</td>
<td>0.47</td>
</tr>
<tr>
<td>III. 1909–1910</td>
<td>3-day cord for all cases.</td>
<td>819</td>
<td>3</td>
<td>5</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Several other institutes are employing very intensive treatments, but their cases are still too few for consideration.

Some directors still use the older methods on the whole and even prolong the treatment. Remlinger, for example, begins with the 9-day cord, and ends according to the three classes of bites respectively in 18, 28, and 30 days.

**Rapid Drying of Rabies Virus.**—Recently Harris, of St. Louis, has published a new method of drying rabies virus and of regulating the dosage.

**Technique.**—The brain and cord are removed aseptically and ground up in a sterile mortar with a sufficient quantity of CO₂ snow thoroughly to freeze the tissue. The frozen nerve tissue and snow are then placed in a Scheibler jar over H₂SO₄, the jar being kept in a frigo apparatus. A vacuum of from 5 to 2 mm. is produced in the jar, which is then kept at the temperature of 18° C. by an ice and salt mixture for a sufficient length of time to dry thoroughly the nerve substance, which then appears as a dry powder. About two days are required for one brain and cord.
which lose about one-half of their virulence in the process. The powder is then sealed in tubes in vacuo and kept at a temperature below 0° C. until required for use.

It has been found that by keeping the powder thoroughly dry and cold practically no further loss of virulence occurs for at least six months.

Before storing the virus for use its strength in units is computed, the unit being the minimal infecting dose (M. I. D.) for a rabbit when injected intracerebrally.

The advantages claimed for this method are: (1) the ease and economy with which a large amount of virus can be prepared, it being necessary to prepare the virus for use even in large laboratories only at intervals of several months; (2) the possibility of more accurate dosage for the patients; (3) a shortened period of treatment; and (4) the inoculation of more virus units.

The required amount of powdered virus is weighed out each morning, and the necessary dilutions in salt solution for the various patients are made from this.

No cases of paralysis have been reported, but we must wait for further statistics before being able to judge of the efficiency of this method in human beings. As far as animal experiments show this method, according to Poor, promises well.

**Fixed Virus Attenuated by Heat.**—This method was first used by Babes in Roumania, and it is still a part of the complicated Roumanian method (p. 732).

It has been used since 1896 by Puscariu, of Jassey. Simon, who reports personal communications from Puscariu, divides the latter’s methods of treatment into three periods.

1. From 1891 to 1896 Babes’ modification of the Pasteur treatment was used. Six hundred and thirty-one cases were treated, with 7 deaths.

2. From 1896 to 1901 Puscariu’s technique was employed, which was as follows: The brain of a rabbit infected with fixed virus was ground up with 100 c. c. normal salt solution in a sterile mortar and strained through a fine sieve. It was then placed in test tubes and heated in a special water bath for 15 minutes at different temperatures for the different days. During the above period the emulsions were heated from 80° to 45° C., the dose was 2 to 3 gm. daily, and two injections were given each day.

The duration of the treatment was from 12 to 21 days.

Two thousand six hundred and thirteen cases have been treated, with 10 cases of paralysis and a mortality of 0.4 per cent.

3. From 1901 to the present time a less intensive scheme has been used. The emulsions heated from 80° to 70° C. have been omitted, and only one injection each day has been given. In 1912 Puscariu reported
that 3,000 cases had been treated by the above scheme, without a death from rabies and without a case of paralysis.

The schema of heating used in this present method at Jassey is as follows:

<table>
<thead>
<tr>
<th>Day</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light cases</td>
<td>65°</td>
<td>60°</td>
<td>55°</td>
<td>65°</td>
<td>60°</td>
<td>55°</td>
<td>50°</td>
<td>45°</td>
<td>Fixed virus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium cases</td>
<td>65°</td>
<td>60°</td>
<td>55°</td>
<td>60°</td>
<td>60°</td>
<td>55°</td>
<td>50°</td>
<td>45°</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe cases</td>
<td>65°</td>
<td>60°</td>
<td>55°</td>
<td>60°</td>
<td>60°</td>
<td>55°</td>
<td>50°</td>
<td>45°</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

As long as we do not know the site and severity of the bites, the time intervening between bites and beginning treatment, the diagnosis of the animals biting, and other details mentioned on pages 737 and 738, we cannot judge how much these results mean.

It has been claimed by others, judging from the earlier results obtained with the heat method, that this treatment produces more cases of paralysis. Babes himself says that he had more cases of paralysis, but fewer cases of death.

**Other Methods of Attenuating the Virus for Dosage.**—The methods by partial digestion and by bile have been recommended, but have not been used to any extent in practice.

**Attenuation by Glycerin.**—Calmette recommends for beginning inoculations a fixed virus cord that has been kept in glycerin until it has lost its virulence (from 3 to 5 months). The method of preserving in glycerin the cords dried by the Pasteur method has also been used. This is well in small institutes with few patients, as in Bern. Up to the end of 1912 187 persons were treated by this method in Bern, with no deaths. This method is also used to send treatment by mail (see p. 726).

**Attenuation by Carabolic Acid.**—Fermi, of Sarossarsi, began using the following method in 1900: A 5 per cent. emulsion of fixed virus in normal salt solution is sterilized by 1 per cent. carabolic acid. Three c. c. are given each morning and each evening over a period of 25 to 30 days. Between 1900 and 1908 1,053 persons were treated, with two deaths. Since 1907 Fermi has used a serum-vaccine mixture, but not according to Marie's method. The carbolized vaccine and antirabic horse serum are mixed in equal amounts and allowed to stand for an hour. Three c. c. are injected daily. Fermi does not titrate his serum, and he apparently pays no attention to the possible excess of serum in the mixture, hence we cannot give an opinion of the superiority of this method over Marie's.

**Fixed Virus Modified by Dialysis.**—Cumming, of Ann Arbor, has devised a method of antirabic vaccination, by which he uses fixed virus which has been rendered avirulent by dialysis. The emulsion of fixed virus is placed in collodion sacs (prepared by the Novy method and stcr-
lized in the autoclave at 105° C. for 20 minutes) and dialysed in distilled water for from 12 to 24 hours. The resulting vaccine does not produce rabies on intracranial inoculations, but does produce immunity on subcutaneous inoculations. Experiments by Cumming on rabbits show that whereas the original Pasteur method protects against only twice the minimum lethal dose (minute directions for obtaining the M. L. D. are given) injected intracerebrally, and the Högyses method against one and one-half times the fatal dose, the dialysis method protects against at least three times the fatal dose. He also claims that immunity is produced at an earlier date than by the other methods. Treatment (2 c. c. of the vaccine) is given daily for from 15 to 25 days. Cumming reports over 800 cases (62 per cent. bitten by animals proved to have been rabid) treated without a death and without complications.

Poor, experimenting on animals with this method, reports results comparing favorably with those of the Harris method.

Method in Which Fresh Fixed Virus Is Used for Inoculations.—(1) Doses Regulated by Dilutions. Högyses’ Method.—The brain of a rabbit dying after fixed virus infection is rubbed up with 100 parts of a 0.7 per cent. salt solution. This is the original mixture from which the dilutions to be used in the inoculations are made. These dilutions are 1-200, 1-500, 1-1000, and 1-2000. The doses given are 1½ to 4 c. c., which represent 0.001 to 0.04 gm. of cord. According to Simon, the schemata of Högyses’ method, which at first were more complicated, may now be condensed as follows:

<table>
<thead>
<tr>
<th>Day</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
<th>17</th>
<th>18</th>
<th>19</th>
<th>20</th>
<th>21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slight cases—children</td>
<td>0.001</td>
<td>0.002</td>
<td>0.003</td>
<td>0.004</td>
<td>0.005</td>
<td>0.0075</td>
<td>0.01</td>
<td>0.015</td>
<td>0.02</td>
<td>0.025</td>
<td>0.025</td>
<td>0.03</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium cases</td>
<td>0.002</td>
<td>0.003</td>
<td>0.004</td>
<td>0.005</td>
<td>0.01</td>
<td>0.015</td>
<td>0.02</td>
<td>0.025</td>
<td>0.03</td>
<td>0.035</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very severe cases</td>
<td>0.002</td>
<td>0.004</td>
<td>0.006</td>
<td>0.008</td>
<td>0.01</td>
<td>0.015</td>
<td>0.02</td>
<td>0.025</td>
<td>0.03</td>
<td>0.035</td>
<td>0.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This method has been used in Budapest since 1890. Simon reports 45,477 cases with 2 paralyses and 131 deaths. A markedly good effect from the Högyses method appears in the statistics from Welkevedren as quoted by Borger (Simon). Up to 1906 the intensive method of Pasteur was used with the following results:

1,379 Europeans treated with 10 cases of paralyses......1-138
2,073 Islanders treated with 1 case of paralysis.........1-2073
TREATMENT

After 1906 Høgyes' method was used with the following results:

751 Europeans treated with 1 case of paralysis........1-751
2,189 Islanders treated with 0 case of paralysis.

In Madrid, also, Høgyes' method is used. Marillo in 1912 reported
3,000 cases treated, with no case of paralysis. The same method has been
adopted in India. In this country Rambaud has used this method for
several years, but he does not compare his results with those by his earlier
Pasteur method, neither does he mention paralysis. Since 1908 he had
used, following Marie, a combined serum and vaccine treatment. For
the last three years he gives an average corrected mortality of 0.14 per
cent.

The advantages of this method seem to be its simplicity, its inexpen-
siveness, and, above all, its claimed good results.

Superintensive Method.—The use of unmodified fixed virus in
large doses has been given this name. It was advocated and practiced by
Ferrán, of Barcelona, early in the history of the Pasteur treatment. In
his original method Ferrán used comparatively large doses of emulsions
of the fresh fixed virus brain. Ferrán states that he occasionally noted
cases of rabies (as did others in the early days of antirabic treatment)
which seemed to be due to the treatment itself. This he attributed to
small particles of the virulent emulsion carried to the brain by the leuko-
cyes. He then sought for a substance that would be positively chemotac-
tic for the leukocytes and so hold them back. This he found in mercury,
which in combining with the albumin in the virus forms an albuminate
of mercury. Since using this modification he claims that he has excluded
the harmful properties of the treatment without impairing the immunizing
strength to any extent. It should be noted that Bareggi, using the origin-
al method of Ferrán in 1889, had five deaths from paralytic rabies due
to fixed virus infection. The Italian government in consequence forbade
the use of the early Ferrán method.

Fifteen thousand persons have been treated by the modified Ferrán
method, with a mortality ranging from 0.2 to 0.4 per cent. Only one
strength of emulsion is used for all patients, and the treatment lasts five
days. All cases coming for treatment later than ten days after the bite are
refused treatment.

Details of the Treatment.—Eighty ccm. of virulent brains or cord are
emulsified with 2 gm. of sterile sand gently and thoroughly in a mortar.
Eight c. c. of fluid are added drop by drop. This fluid is a mercury
preparation, which with the emulsion forms an albuminate of mercury.
The mixture is allowed to stand one-half hour before decanting the fluid.
This decanted fluid is used for the injections (6 c. c.) which are made
each day in three injections on five consecutive days. In bad cases the
course of treatment is repeated after an interval of from 1 to 10 days.
The treatment causes a moderate local induration sometimes lasting several months. If paralyzes occur they are non-fatal.

Ferrán states that his inoculations should only be made subcutaneously, as cutaneous and intramuscular inoculations may produce infection (1). He claims that large amounts of the virus by the (hypothetical) toxin they contain produce an immunity more quickly than the living rabies germs, and so protect the patient from infection with the vaccine, while small doses of the vaccine might produce rabies.

In this country Proescher, of Pittsburgh, has used a similar method. He concludes that his strain of fixed virus (Pittsburgh) is harmless for human beings because he injected two men each with an entire fixed virus brain intramuscularly without ill effect to them. A control rabbit injected subdurally with a 2 per cent. dilution of the same died in seven days with experimental rabies. He further states that he has used doses 50 times as great as those of Ferrán, with no deaths from rabies infection. In 1911 he reports 92 cases which were treated by injections of unchanged fixed virus.

His technique is as follows: An amount of brain substance averaging from 0.10 to 0.12 gm. is removed by the jaws of a pair of urethral forceps. This is emulsified in 30 c. c. of salt solution. Three c. c. of this emulsion (equal to about 0.01 gm. of fixed virus) is injected subcutaneously. One injection is given each day for five days.

The most important result of the superintensive method is its demonstration of the harmlessness in the majority of people of large subcutaneous doses of fixed virus. However, until we know more of the conditions causing the susceptibility to fresh fixed virus infection which occurs in a small percentage of people, such large doses given at the beginning of treatment should be considered with reserve.

**Serum-vaccine Treatment**

**The Roumanian Method.**—Babes began using antirabic serum as early as 1890. By combining it with the Pasteur method he found that it gave good results in severe bites such as those received from the wolf. He also tried combining the Pasteur method with heated virus. He has gone minutely into the subject of this treatment in his recent book, "Traité de la rage." He insists on individual alterations of treatment. As an example of his treatment of a very severe face wound the schema on page 733.

This elaborate method he simplified in 1906 (1) by beginning with a six-day cord and (2) by giving only one series of heated cords in 10 c. c. doses. The treatment lasts from 20 to 30 days. With this modification he says that, while his absolute mortality from wolf bites remains at 5 to 6 per cent., not one case died after the 15-day limit of observation.
After severe dog bites Babes does not use the heated vaccine, but does add the serum, according to the following schema:

**Dose 3 c. c. of 1-10 Solution**

<table>
<thead>
<tr>
<th>Day</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
<th>17</th>
<th>18</th>
<th>19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cords</td>
<td>Light</td>
<td>6</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>6</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Six thousand five hundred and twenty-five cases have been treated by this method, with 8 paralyses and a total mortality of 0.452.

**Marie's Method.**—For several years past the use of virus-serum mixture has been in vogue at the Pasteur Institute in Paris, the technique of which is as follows: 1 gm. of the medulla of a rabbit dead of fixed virus is finely emulsified with 9 c. c. of .8 per cent. salt solution and filtered through linen. Two c. c. of this emulsion and 4 c. c. of antirabic serum (obtained from sheep, and inactivated at 56° C. for 30 minutes) are carefully mixed after standing for a time. Six c. c. of this mixture, which contains an excess of virus, is injected into the patient. These injections are repeated on the next three days, after which the treatment proceeds according to the regular Pasteur schema, beginning with the use of a 6-day cord on the 5th day. The antirabic serum is obtained from sheep which have been subjected to a long and strong course of treatment with fixed virus. It is claimed that a quicker immunity is produced by the serum-virus mixture than by the original Pasteur scheme, an advantage of especial value in the treatment of cases liable to become infected with a short incubation, such as bites on the head. Three thousand nine hundred and ninety-three cases are reported treated by this method, with a mortality of 0.23 per cent.
Antirabic Serum

The possibility that the serum of animals immunized against rabies contains protective substances was suggested by Pasteur as early as 1889. The following year Babes recommended the use of the serum of vaccinated animals in combination with the Pasteur treatment. Since then the study of the amount and character of the antibody content of animals immunized against rabies has been carried on more or less extensively both from the theoretic and the practical sides. It was hoped that a serum could be obtained that would effect a cure for developed rabies just as diphtheria antitoxin does for developed diphtheria. But such a definite applicability of the serum has not developed. It was soon found that, while serum of certain vaccinated animals possessed the property of neutralizing rabies virus in vitro, it had only a slight inhibiting power when inoculated into the living animal, and apparently no action at all by any method of inoculation after the disease had become manifest. Babes still claims, however, that the serum has enough effect in vivo to be used in treatment, and his serum treatment is based upon this claim. He gives as his reason for employing serum at the end of treatment that he wishes to introduce into the patient at the time he most needs it the largest amount of antibodies. He also claims that the serum so given will prevent or cure the occasional paralyses which occur during treatment.

Those who did not agree with Babes were led to test the practical use of the serum combined with the beginning vaccine inoculations.

Remlinger, Marie, and others showed that a serum-virus mixture with a slight excess of virus will protect an animal against infection into the anterior chamber of the eye when inoculated during the three days following the vaccination. Thus he showed that immunity is produced more quickly by these unsaturated mixtures of virus and serum than by the virus alone. If a surplus of serum is present the animals are not protected from a later infection. The results of Marie's method of inoculating dogs with only one injection of an unsaturated virus-serum mixture is shown by the following table:

<table>
<thead>
<tr>
<th>Dog</th>
<th>Date of Injection</th>
<th>Amount Injected</th>
<th>Date of Infection</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dec. 16</td>
<td>10 c. c.</td>
<td>Jan. 23</td>
<td>Living</td>
</tr>
<tr>
<td>2</td>
<td>Dec. 16</td>
<td>10 c. c.</td>
<td>Jan. 23</td>
<td>Living</td>
</tr>
<tr>
<td>3</td>
<td>Dec. 16</td>
<td>10 c. c.</td>
<td>Jan. 23</td>
<td>Living</td>
</tr>
<tr>
<td>4</td>
<td>Feb. 25</td>
<td>8 c. c.</td>
<td>Mar. 21</td>
<td>Rabies I-IV</td>
</tr>
<tr>
<td>5</td>
<td>Aug. 11</td>
<td>10 c. c.</td>
<td>Sept. 14</td>
<td>Living</td>
</tr>
</tbody>
</table>

A serum containing such properties is only found in animals that have undergone a protracted series of inoculations of gradually increas-
ing strength. Marie, who has used the serum in humans since 1904, prepares it as follows: The brain of two rabbits dying from fixed virus infection are finely rubbed up with physiologic salt solution in the proportion of 20 gm. in 180 c. c. This emulsion is filtered through fine cloth and heated for one-half hour at 37° C. Sheep are used for the inoculation. Each sheep receives intravenously 30 c. c. (3 gm. fixed virus) a week for 6 to 8 weeks. Thirteen days after the last inoculation the first blood is drawn. Then in a period of two weeks, at 4 bleedings, 200 c. c. of blood are drawn. After a 14-day pause another series of inoculations are given and the animal is ready for another series of bleedings. From each animal yearly about 3 l. of antirabic serum are obtained.

Remlinger's method of inoculating sheep is to begin with 3 or 4 intravenous inoculations of fixed virus, and then to go on with subcutaneous inoculations until finally an entire fixed virus brain in 400 c. c. of normal salt solution has been inoculated.

The dose of the inoculated antigen is of importance in producing a high-grade serum. Smaller doses than those given above produce weaker serums, according to Tizzoni and Centanni, Marie and others. A strong serum is one that neutralizes 40 virus units in 1 c. c.

A virus unit is 1 c. c. of 5 times the dilution of fixed virus that will surely kill a rabbit inoculated intracerebrally, e. g., the unit of a fixed virus that will surely kill a rabbit in 1-500 dilution is 1 c. c. of a 1-100 dilution.

<table>
<thead>
<tr>
<th>Animals</th>
<th>Amount of Serum in c. c.</th>
<th>Amount of Virus in c. c.</th>
<th>Time Inoculations Made After Mixture</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Immediately</td>
<td>24 hrs.</td>
</tr>
<tr>
<td>Sheep</td>
<td>0.1</td>
<td>1.0 Dil. 1:100</td>
<td>+</td>
<td>Living</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>1.0 Dil. 1:100</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>1:10 0.1 (not filtered) Dil.</td>
<td>+</td>
<td>Dead (rabies)</td>
</tr>
<tr>
<td>Dog I</td>
<td>0.05</td>
<td>1.0 Dil. 1:100</td>
<td>+</td>
<td>Living</td>
</tr>
<tr>
<td></td>
<td>0.1 heated to 58° C.</td>
<td>1.0 Dil. 1:100</td>
<td>+</td>
<td>Living</td>
</tr>
<tr>
<td>Dog II</td>
<td>0.05</td>
<td>1.0 Dil. 1:100</td>
<td>+</td>
<td>Living</td>
</tr>
<tr>
<td></td>
<td>0.05 heated to 60°C for ½ hr.</td>
<td>1.0 Dil. 1:100</td>
<td>+</td>
<td>Living</td>
</tr>
<tr>
<td>Horse</td>
<td>0.01</td>
<td>1.0 Dil. 1:100</td>
<td>+</td>
<td>Living</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>1.0 Dil. 1:100</td>
<td>+</td>
<td>Living</td>
</tr>
</tbody>
</table>
The demonstration of antibody content of the serum may be shown by the table (see page 735) of Krauss (Heller and Rothermundt in Kolle and Wassermann, 1913).

The nature of the antibodies in rabies serum has been the subject of many studies. Fermi and a few others claim that the antibodies are not specific. They say that they can obtain a similar serum after the inoculation of normal brain emulsions. Some even use normal brain emulsions in the treatment of their lighter cases.

Certain investigators (Kraus, Marie, and others), while not able to corroborate all of these claims, have found that the serum of certain animals which are more or less refractory to rabies possesses a small amount of rabicidal strength; e.g., 0.5 c. c. of normal chicken serum mixed with one unit of fixed virus (1 c. c. of 1-100 dilution) causes the latter to become neutral in 18 hours.

The neutralizing property is not due to a neurotoxic substance since animals stand very large doses of the serum without harm.

All species of animals tried produce the specific antibodies, but not to an equal degree. Human beings and monkeys are said to have more antibodies after vaccination than rabbits.

<table>
<thead>
<tr>
<th>Year</th>
<th>Cases Treated</th>
<th>Number of Deaths</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1886</td>
<td>2,671</td>
<td>25</td>
<td>0.94</td>
</tr>
<tr>
<td>1887</td>
<td>2,770</td>
<td>14</td>
<td>0.79</td>
</tr>
<tr>
<td>1888</td>
<td>1,062</td>
<td>9</td>
<td>0.55</td>
</tr>
<tr>
<td>1889</td>
<td>1,830</td>
<td>7</td>
<td>0.38</td>
</tr>
<tr>
<td>1890</td>
<td>1,540</td>
<td>5</td>
<td>0.32</td>
</tr>
<tr>
<td>1891</td>
<td>1,550</td>
<td>4</td>
<td>0.25</td>
</tr>
<tr>
<td>1892</td>
<td>1,790</td>
<td>4</td>
<td>0.22</td>
</tr>
<tr>
<td>1893</td>
<td>1,648</td>
<td>6</td>
<td>0.36</td>
</tr>
<tr>
<td>1894</td>
<td>1,387</td>
<td>7</td>
<td>0.50</td>
</tr>
<tr>
<td>1895</td>
<td>1,520</td>
<td>5</td>
<td>0.38</td>
</tr>
<tr>
<td>1896</td>
<td>1,308</td>
<td>4</td>
<td>0.30</td>
</tr>
<tr>
<td>1897</td>
<td>1,529</td>
<td>6</td>
<td>0.39</td>
</tr>
<tr>
<td>1898</td>
<td>1,465</td>
<td>3</td>
<td>0.20</td>
</tr>
<tr>
<td>1899</td>
<td>1,614</td>
<td>4</td>
<td>0.25</td>
</tr>
<tr>
<td>1900</td>
<td>1,420</td>
<td>4</td>
<td>0.28</td>
</tr>
<tr>
<td>1901</td>
<td>1,321</td>
<td>5</td>
<td>0.38</td>
</tr>
<tr>
<td>1902</td>
<td>1,005</td>
<td>2</td>
<td>0.18</td>
</tr>
<tr>
<td>1903</td>
<td>623</td>
<td>2</td>
<td>0.32</td>
</tr>
<tr>
<td>1904</td>
<td>755</td>
<td>3</td>
<td>0.39</td>
</tr>
<tr>
<td>1905</td>
<td>721</td>
<td>3</td>
<td>0.41</td>
</tr>
<tr>
<td>1906</td>
<td>772</td>
<td>1</td>
<td>0.43</td>
</tr>
<tr>
<td>1907</td>
<td>788</td>
<td>3</td>
<td>0.38</td>
</tr>
<tr>
<td>1908</td>
<td>524</td>
<td>1</td>
<td>0.19</td>
</tr>
<tr>
<td>1909</td>
<td>467</td>
<td>1</td>
<td>0.21</td>
</tr>
<tr>
<td>1910</td>
<td>401</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>1911</td>
<td>341</td>
<td>1</td>
<td>0.29</td>
</tr>
<tr>
<td>1912</td>
<td>395</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>1913</td>
<td>330</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>1914</td>
<td>373</td>
<td>0</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Total ........... 34,492        129        0.35
TREATMENT

Centanni showed that immediately after vaccination the animal is not fully protected, though its serum may contain antirabic qualities, while later the animal is immune, though its serum may not be able to neutralize the rabies virus. These facts point to a cellular immunity.

Results of Antirabic Treatment.—On the whole the results of protective inoculations against rabies are marked. One has only to compare the statistics of mortality after bites from animals suffering from hydrophobia with those given after any of the methods of treatment employed to see the benefit. As regards the best method to use, the case is different. With many methods tried in many lands on a large number of cases, it would seem that we should be able by this time to determine their comparative worth. But the trouble is that the improvement on the whole is not great and the statistics are not kept uniformly or minutely enough to draw trustworthy comparisons. That there has been more or less steady lessening of mortality is shown by such statistics as those given by the Pasteur Institute of Paris from 1886 to 1911. (See page 736.)

A similar slight decrease in mortality has been shown in the statistics from most of the other antirabic institutes of the world.

Babes quotes the following total mortality for cases treated during a space of three years according to different schemes of vaccination:

<table>
<thead>
<tr>
<th>Locality and method</th>
<th>Cases Treated</th>
<th>Mortality, per Cent.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bucharest (Roumanian method)</td>
<td>3,091</td>
<td>0.12</td>
</tr>
<tr>
<td>Paris (Pasteur method)</td>
<td>2,115</td>
<td>0.61</td>
</tr>
<tr>
<td>Berlin (modified Pasteur method)</td>
<td>934</td>
<td>1.28</td>
</tr>
<tr>
<td>Vienna (Pasteur method)</td>
<td>762</td>
<td>1.04</td>
</tr>
<tr>
<td>Budapest (Högyes method)</td>
<td>8,658</td>
<td>0.77</td>
</tr>
</tbody>
</table>

But these figures tell us little about the actual value of the different methods. In order to be able better to judge, the statistics should uniformly give many more details. Some institutes give such details, others do not. Until some such scheme as the following is carried out by all, we must change cautiously a treatment that has already given good results.

(1) Diagnosis of biting animal:
   (a) Rabies, (b) probably rabies, (c) questionable, (d) not rabies, (e) nothing known.

(2) Manner of making diagnosis:
   (a) By animal inoculation, (b) by microscopic examination, (c) by clinical diagnosis.

(3) Site and character of bites (e. g., number, depth, laceration, protected by clothing, etc.).
   (a) Head, (b) hands, (c) other parts of body.
HYDROPHOBIA

(4) Time elapsing between bite and beginning of treatment.
(5) Method of treatment used.
(6) Complications during or after treatment, particularly paralysis.
(7) Character and time of death.

That the time after the bite makes a great difference is shown by the following table:

<table>
<thead>
<tr>
<th>Time Intervening between Bite and Beginning Treatment</th>
<th>Number of cases treated</th>
<th>Death</th>
<th>Percentages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Babes. 1-2 days.</td>
<td>3,406</td>
<td>3</td>
<td>0.088</td>
</tr>
<tr>
<td>3-5 days.</td>
<td>2,541</td>
<td>2</td>
<td>0.077</td>
</tr>
<tr>
<td>5-6 days.</td>
<td>809</td>
<td>1</td>
<td>0.124</td>
</tr>
<tr>
<td>Disto. 1 week.</td>
<td>4,602</td>
<td>26</td>
<td>0.560</td>
</tr>
<tr>
<td>2 weeks.</td>
<td>961</td>
<td>16</td>
<td>1.660</td>
</tr>
<tr>
<td>3 weeks.</td>
<td>313</td>
<td>10</td>
<td>3.190</td>
</tr>
</tbody>
</table>

IMMUNITY.—The immunity in human beings produced by the anti-rabic treatment apparently lasts a variable time. That it may not last more than 14 months is shown by the history of one of our cases.

The patient was an assistant in a hospital for dogs. He was given 18 days’ treatment after a light wound on the hand from a rabid dog.

Fourteen months later he came down with typical hydrophobia. Since his treatment he had become very careless with cases of rabies, exposing wounded hands to saliva because he considered himself immune.

He was warned that there might be danger. Six weeks before his death he put a wounded hand into the mouth of a rabid animal.

There is little doubt but that this is a case of reinfection after loss of protection from the treatment rather than one of delayed hydrophobia.

Marie has found complete immunity in dogs 18 months after treatment.

Contraindications for Treatment.—No obvious contraindications exist. That extremely few people have an individual susceptibility from unknown causes is probable. The results of this condition are taken up in the next section.

Ill Effects for Treatment.—Local.—There is only slight local discomfort, increased a little if the emulsion contains glycerin. During the second week an erythema often appears about the point of inoculation, which Stimson regards as a manifestation of hypersusceptibility to foreign nerve tissue. It disappears in a few days.
TREATMENT

CONSTITUTIONAL.—Ever since the beginning of treatment occasional non-fatal affections of the nervous system have been reported, which occurred during or shortly after the course of treatment. These have varied in degree all the way from a slight neuritis, through paraplegia to paralysis of various parts of the body. Very occasionally the paralyses are marked and the patient dies. Cases of true paralytic rabies which may occur within the period required for the establishment of immunity by the treatment must be differentiated from cases occurring as a result of treatment.

Remlinger in 1905 collected the cases of this character so far published. Poor in 1908 published the few occurring among the many treated by the New York City Health Department, and recently (February, 1913) Simon published an extensive report of 84 cases occurring during the years 1888 to 1911 inclusive, and mentions a few others about which he could not get sufficient data.

The following table which he gives we copy in order to show the small percentage of these cases that have occurred during the whole time the treatment has been used up to 1912:

<table>
<thead>
<tr>
<th>Name of Institute</th>
<th>Number of Cases</th>
<th>Name of Institute</th>
<th>Number of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Paralytic</td>
<td>Treated</td>
<td></td>
</tr>
<tr>
<td>Berlin</td>
<td>4</td>
<td>4,221</td>
<td>Kasan</td>
</tr>
<tr>
<td>Breslau</td>
<td>2</td>
<td>985</td>
<td>Wilna</td>
</tr>
<tr>
<td>Paris</td>
<td>6</td>
<td>32,045</td>
<td>Charkow</td>
</tr>
<tr>
<td>Algiers</td>
<td>2</td>
<td>4,755</td>
<td>St. Petersburg</td>
</tr>
<tr>
<td>Milan</td>
<td>6</td>
<td>2,942</td>
<td>Athens</td>
</tr>
<tr>
<td>Bologna</td>
<td>6</td>
<td>3,062</td>
<td>Constantinople</td>
</tr>
<tr>
<td>Naples</td>
<td>2</td>
<td>4,578</td>
<td>Weltwerden</td>
</tr>
<tr>
<td>Trente</td>
<td>1</td>
<td>1,440</td>
<td>Florence</td>
</tr>
<tr>
<td>Turin</td>
<td>2</td>
<td>2,207</td>
<td>Madrid</td>
</tr>
<tr>
<td>Palermo</td>
<td>4</td>
<td>7,129</td>
<td></td>
</tr>
<tr>
<td>Barcelona</td>
<td>3</td>
<td>1,784</td>
<td></td>
</tr>
<tr>
<td>Lisbon</td>
<td>1</td>
<td>12,888</td>
<td></td>
</tr>
<tr>
<td>Budapest</td>
<td>2</td>
<td>49,382</td>
<td></td>
</tr>
<tr>
<td>Krakau</td>
<td>2</td>
<td>1,424</td>
<td></td>
</tr>
<tr>
<td>Bucharest</td>
<td>15</td>
<td>7,056</td>
<td></td>
</tr>
<tr>
<td>Jassey</td>
<td>10</td>
<td>5,458</td>
<td></td>
</tr>
</tbody>
</table>

Total 100 211,774

Only 1 in 2,117 cases, or 0.048 per cent. As less than one-fourth of these have been fatal, including those cases known to have resulted from fixed virus infection, the total mortality is less than 1 in 10,000.

This table does not include the 7 cases from the New York City Health Department or the 3 reported from the Hygienic Laboratory at Washington. A number of these cases have occurred in those receiving the treatment, but not bitten by rabid animals.
Simon classifies the cases collected by him according to the diagnosis of the biting animal, with the mortality in each group as follows:

<table>
<thead>
<tr>
<th>Positive Group</th>
<th>Probable Group</th>
<th>Questionable Group</th>
<th>Negative Group</th>
<th>Not Known Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number Cases</td>
<td>Per Cent.</td>
<td>Number Cases</td>
<td>Per Cent.</td>
<td>Number Cases</td>
</tr>
<tr>
<td>25 (27)</td>
<td>20.78</td>
<td>11 (47)</td>
<td>13.0</td>
<td>21 (57)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25 (27)</td>
<td>25</td>
<td>17 (35)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10 (20)</td>
</tr>
</tbody>
</table>

Nineteen deaths occurred, as seen from the figures in parentheses, or 22 per cent. of the 84 cases.

In analyzing the effect of different methods of treatment on paralyces, Simon gives the following summary:

<table>
<thead>
<tr>
<th>Method</th>
<th>Number of Cases Treated</th>
<th>Cases of Paralyces</th>
<th>Proportion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classical Pasteur method</td>
<td>32,676</td>
<td>6</td>
<td>1:5446</td>
</tr>
<tr>
<td>Modified Pasteur method</td>
<td>8,657</td>
<td>16</td>
<td>1:541</td>
</tr>
<tr>
<td>Högyes method</td>
<td>51,417</td>
<td>3</td>
<td>1:17139</td>
</tr>
</tbody>
</table>

It is seen that the number of paralyces following the Högyes method are markedly less than those following the other methods.

From the studies so far made of these paralyces the possibility of there being different causes for different cases cannot yet be ruled out. The chief theories advanced as to factors in producing the condition are six:

1. Due to "laboratory rabies" from the fixed virus vaccine inoculated.
2. Due to "modified rabies" resulting from the treatment on the street virus infection.
3. Due to a toxin produced by the rabies organisms.
4. Due to infection with extraneous organisms introduced with the virus during treatment.
5. Due to psychologic disorders.
6. Due to the inoculation of a foreign proteid with a subsequent anaphylactic reaction.

Simon includes Bereggi's five cases of undoubted fixed virus infection. These were cases that had been inoculated with large doses of unmodified fixed virus, and test-animal inoculations showed fixed virus infection. These cases must have had a special predisposition or the virus must have been especially virulent, since many cases in different parts of the world have been inoculated with large amounts of unmodified fixed virus and have shown no symptoms.
REFERENCES

One of the other fatal cases (following the Berlin intensive method) that was tested showed fixed virus in his brain, and one showed street virus infection. Hence the first and second theories cannot yet be ruled out as factors in at least a few of the cases. Five of the cases tested showed no rabies virus in their brains, therefore the third or the sixth theory may be applicable to them. Unfortunately seven of the cases were not tested. The fourth and the fifth theories may also be ruled out, since, if ever applicable, they would be so only in very infrequent, unimportant, non-fatal cases, as is shown in some of those that Poor reported.

We may conclude that the third and the sixth theories embrace the two most probable factors in the majority of the cases of paralysis.

Treatment of the developed disease, which is simply palliative and non-specific, is considered in Vol. II.

Summary of Present State of Specific Treatment of Hydrophobia

(1) The specific vaccine treatment by attenuated virus or by dilutions of fresh virus protects the great majority of the cases that begin treatment immediately after the infection; the very few unprotected ones are among those who have been bitten very severely or who have been infected with an unusually virulent virus or who are peculiarly susceptible.

(2) Antirabic serum alone possesses neither a protective nor a curative action; combined with the vaccine so that the latter is not completely saturated, the mixture seems to produce a quicker and stronger immunity.

(3) The comparative worth of the many methods advocated for the preventive treatment of rabies cannot be positively determined until standard rules for the recording of statistics are adopted.

REFERENCES

Babes, V. Traité de la rage, 1st ed., Paris, 1912: (With Bibliography.)
Heller and Rothermundt. Wutschutzimpfung und Wutimmunität. Kolle
HYDROPHOBIA

und Wassermann's Handb. der path. Mikroor., 2nd ed., Jena, 1913. (With Bibliography.)

Högyes. Lyssa, Wien, 1897. (With Bibliography.)


Negri Bodies with Reference to Diagnosis. Ibid., 1906, v, 155.

Studies in Hydrophobia. Ibid., 1906, vi, 77.


CHAPTER XXXIV

SNAKE VENOMS AND ANTISERA

WILLARD J. STONE

HISTORICAL

Wolfenden (31) in 1886 published an article upon the action of venom of poisonous snakes, which was followed in 1887 by an article by Sewall (25). One of the early attempts to separate the toxins present in snake venoms was made by Mitchell and Reichert (20). They were able by dialysis to separate from the venom of Crotalus adamanteus (rattlesnake) a precipitate, or "globulin fraction," and a portion not precipitated, or "peptone fraction." The globulin fraction was found to possess hemolytic powers, while the peptone fraction possessed greater neurotoxic activity. Much of the early work on the toxic constituents of venoms was also done by Calmette (5), Fraser (12), Ewing (9), Stephens (26, 27), Myers (21, 27), Flexner (11), Noguchi (11, 22), Kyes (14, 15), Sachs (15, 24), Abderhalden and Le Count (1, 2), and Goebel (13). Additional papers by Noguchi, von Dungern (7), and Coca (6, 7), Kyes, Manwaring (17), and Bang (3) during the past six years upon the subject of venom hemolysis have created new interest in the subject.

THE TOXIC CONSTITUENTS OF VENOMS

The toxic properties of different snake venoms depend upon the presence in varying amounts of (1) hemagglutinins, (2) hemolysins, (3) hemorrhagin, (4) neurotoxin.

The hemagglutinins and hemolysins act upon the red blood cells. The hemagglutinins are supposed to agglutinate the cells prior to hemolysis, although McFarland and Weston (16) found that the agglutination of corpuscles took place independently of hemolysis. The venom of the cobra (Naja tripudians) is remarkably hemolytic for the red blood corpuscles of man. The red cells of the sheep, ox, or goat are not susceptible to strong solutions of cobra venom under natural conditions. Nor are such red cells
susceptible to venom hemolysis when suspended in physiological salt solution. The red cells of these species, however, become highly susceptible when suspended in physiological sugar solutions, as was shown by Goebel and by von Dungern. In their study of venom hemolysins, Flexner and Noguchi found rattlesnake venom to contain little of this poison. They also found that the hemolytic activity of most venoms was greater than their agglutinative power, and that the agglutinating power was destroyed by heating to 75° to 80° C. for thirty minutes. The hemolytic activity was not destroyed at this temperature.

Hemorrhagin acts upon the endothelium of the blood vessels. The toxicity of the venom of the copperhead (Ancistrodon contortrix), water moccasin (Ancistrodon piscivorus), and rattlesnake (Crotalus adamanteus) depends largely upon the action of the hemorrhagin. The venoms of the cobra and water moccasin are also neurotoxic, that is, they act upon the cells of the central nervous system, producing death by paralysis of the respiratory and cardiac centers. Noguchi found that cold-blooded animals were more susceptible to neurotoxin than to hemorrhagin. The following résumé sums up the chief poisonous action of the venoms of some of the common snakes:

Cobra venom is actively hemolytic and contains neurotoxin.
Rattlesnake venom contains hemorrhagin and is slightly hemolytic.
Copperhead venom contains hemorrhagin.
Water-moccasin venom contains hemorrhagin and neurotoxin.

The toxins of venoms are quickly absorbed when injected into the animal body, and poisonous symptoms are very rapidly manifested. Calmette found that inoculating cobra venom into the tip of the tail of a rat produced symptoms of poisoning almost immediately, and that the animal could not be saved by amputating the tail one minute later.

When the venom of poisonous serpents is injected subcutaneously extensive subcutaneous hemorrhage and tissue necrosis is produced at the site of injection, with ecchymoses in other parts of the body, affecting particularly the serous membranes. The blood does not, as a rule, coagulate after death. Following inoculation of lethal doses of cobra or water-moccasin venom dyspnea and motor paralysis promptly follow, with death from paralysis of the respiratory and cardiac centers.

The separation of the hemolytic from the neurotoxic constituents of cobra venom was accomplished by Kyes (loc. cit.). He added a solution of lecithin in chloroform to an aqueous solution of venom and found, after shaking the mixture, that the aqueous venom solution had lost its hemolytic power, while its neurotoxic power was unchanged. Von Dungern (loc. cit.) was able to demonstrate that the chloroform-lecithin portion, after shaking with a solution of cobra venom, contained the hemolytic toxin. Manwaring (loc. cit.) corroborated these observations in 1910, and similar results were secured by Coca in 1912. Coca found that 99 per cent. of
the hemolysin had been absorbed by the lecithin, while the neurotoxin remained unchanged in the aqueous venom solution. It thus seems established that the venom of cobra contains two separable toxic constituents. To Kyes belongs the credit for the recognition of the part played by lecithin in the process of hemolysis. Since lecithin, with a small amount of cholesterin, is a constituent of the stromata of red blood cells, the incomplete hemolysin present in venom unites, according to Kyes, with the lecithin to form the complete hemolysin designated by him “cobralecithid.”

The complete hemolysin is formed from the union of cobra venom with lecithin, and is due to a splitting of the fatty acid radical from the lecithin. Von Dungern, Coca, and Manwaring have contended that the hemolysin was a venom-free lecithin derivative, and not a lecithid. They have preferred to call the active principle, which was shown by von Dungern to be a lipolytic ferment, “cobralecithinase,” and the hemolytic end-product, monofatty-acid-lecithin, or “desoleolecithin.” According to Pasucci (23), the stromata of red blood cells consist of one-third lecithin and cholesterol and two-thirds protein substances. Wells (30) has stated that dried red blood cells contain, according to Hoppe-Seyler, lecithin 0.3 to 0.7 per cent., and cholesterol 0.2 to 0.3 per cent.

It has been stated by Abderhalden (1) that if red cells are washed absolutely free from serums hemolysis does not occur with cobra venom. He does not mention the species of cells or dilutions of venom employed. He found that if a very small amount of serum or lecithin was added hemolysis took place. Human red blood cells contain, at least under normal conditions, sufficient lecithin for the union with cobra venom and the production of hemolysis in the absence of serum. Thus if to a 4 per cent. suspension of human red blood cells in physiological salt solution, the cell of which have been in contact with a 2 per cent. solution of sodium citrate, to prevent clotting, and from which the citrated serum has subsequently been removed by thorough washing, an equal quantity of cobra venom solution in dilutions as high as 1-40,000 to 1-50,000 is added, hemolysis takes place within 24 hours.

COBRA VENOM SOLUTIONS IN THE DIAGNOSIS OF SYPHILIS AND TUBERCULOSIS

Weil (29) has utilized the apparent resistance of the red cells of luetic individuals to cobra venom dilutions 1-15,000 to 1-30,000 as a reaction of diagnostic importance in syphilis. The resistance of luetic red cells to cobra venom hemolysis in these dilutions has been considered to be due to (1) a decreased lecithin cell content in syphilis, or (2) that the red cells had become lecithin-fast, with the result that less lecithin was available in
a free state for the union with cobra venom, or (3) that the choles
erin of lectic red cells was increased in syphilis, which inhibited hemolysis.

In an article on this subject in 1912 by Schottstaedt and myself (28)
the resistance of lectic red cells to cobra venom hemolysis was discussed,
together with the results obtained in a study of 87 patients with syphilis
and 43 normal and controls. Since then the reaction has been studied in
about 150 additional patients. We have reached the following con-
clusions: (1) While we have never found marked red cell resistance
to cobra venom hemolysis, except in lectic individuals, we are con-
vinced that the red cells of some individuals with syphilis hemolyze in a
manner similar to normal cells. (2) The reaction of resistance with cobra
venom dilutions concerned in the test gives readings in syphilis nearly as
high as the Wassermann reaction. (3) The resistance to hemolysis dis-
ppears under antilectic therapy, and as such is of importance as an index
to further treatment.

Field (10) has reported the results obtained in a comparative study
of the Wassermann and Weil cobra venom reactions in syphilis as follows:

<table>
<thead>
<tr>
<th></th>
<th>Primary (per cent.)</th>
<th>Secondary (per cent.)</th>
<th>Tertiary (per cent.)</th>
<th>Questionable and negative cases (per cent.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wassermann positive</td>
<td>70</td>
<td>87</td>
<td>75</td>
<td>30</td>
</tr>
<tr>
<td>Weil cobra venom positive</td>
<td>76</td>
<td>75</td>
<td>73</td>
<td>40</td>
</tr>
</tbody>
</table>

The reaction is of scientific interest as a biochemical phenomenon, as
is also the fact that the red cells of individuals in an active stage of
tuberculosis (and probably in some fevers) possess hypersusceptibility to
hemolysis with cobra venom solutions. This was noted by us in the study
of cobra venom hemolysis in a series of patients with tuberculosis, and
was described in the article mentioned above. Since, with the dilutions
of cobra venom employed in the test, lectic red cells are usually hyposus-
ceptible, while the red cells of tuberculous patients are hypersusceptible,
the reaction is of importance in differentiating syphilis from early tuber-
culosis of the lungs. From the study of this subject it appears that the
lechithin of red cells in active tuberculosis is rendered more available in a
free state for hemolysis with cobra venom as a result of the circulation of
tuberculo-proteins in the blood stream.

**ANTISERA**

It has been stated by Bolduan (4) that in India more than 20,000
persons die annually from the bite of the hooded cobra. It was but nat-
ural that attempts should be made to protect those who suffer inoculation. One of the earliest researches was by Sewall, who showed that it was possible to secure an increased resistance in pigeons to the venom of Crotalophorus tergeminus by repeated inoculations, until seven times the fatal dose produced no harmful effect. It was noted by Sewall that the immunity decreased if the inoculations were discontinued. Calmette was also able to secure immunity to large lethal doses in rabbits by repeated injections of gradually increasing amounts of cobra venom. He started with about one-twentieth of a fatal dose. When the animal was able to withstand 40 mg., or 80 fatal doses, without any reaction, he found that 5 drops of the animal's blood serum were capable of neutralizing 1 mg. of cobra toxin. Calmette called this antiserum "antivenin." Flexner and Noguchi found that hemolysis was prevented by neutralization of the toxic constituents of venom with antivenin, i. e., that in a mixture of 1 c. c. of antivenin with 1 mg. of venom, hemolysis was prevented. Fraser was able to secure some degree of immunity in animals by introducing the venom into the stomach. He found that enormous doses of venom administered in this manner did not produce toxic effects. He also noted that venomous serpents possess definite protective substances in their own blood sera which protect them, not only against their own venom, but also against the venom of other serpents. The protective power of the blood serum of such serpents was found to be decidedly less potent than the sera of animals artificially immunized. Cobra antivenin protects against the neurotoxic constituents of other venoms, which produce death by paralysis of the respiratory and cardiac centers, such as the water-moccasin, the scorpion, and certain poisonous eels. It also contains an antihemolysin which protects against the hemolytic effects of the venoms of other species. It does not protect against the hemorrhagin of the rattlesnake.

Rattlesnake antivenin may be prepared by immunizing animals to the attenuated venom of this species. Goats or horses are usually used. Such antiserum contains antihemorrhagin, and would protect against the venom of the copperhead, but since it contains no protective substances antagonistic to neurotoxin it would not protect against the venom of water-moccasin, cobra, or viper.

It has been estimated that the fatal dose of cobra venom for man is about 15 mg. When lethal doses are inoculated death occurs in from 3 to 24 hours. A dose of venom insufficient to cause death usually produces within a few hours a widespread area of necrosis about the site of inoculation. To be effective antivenin must be used soon after the bite of the serpent, that is, within an hour or two. Fraser found that antivenin produced protection in animals when injected thirty minutes after the venom inoculation.

Since it cannot be determined with any degree of accuracy how much venom has been inoculated at the time of the serpent's bite it would seem
wise if obtainable to employ an excess of antivenin in treatment. It has been shown that mixtures of 1 c. c. of antivenin and 1 mg. of venom are innocuous. It is not probable in single bites that more than 10 mg. are inoculated, which would call for an injection of at least 10 c. c. of the corresponding antivenin. In multiple bites the danger would be correspondingly greater, and larger doses of the antiserum should be used.

Cobra antivenin is usually prepared from the blood serum of immunized horses, and is marketed in two forms, the liquid and the dry. The latter consists of blood serum dried in vacuo, by means of which its antitodal properties are preserved in stable form. It is dissolved in water just before subcutaneous injection. Information is not at hand concerning the use of antivenins intravenously, but, judging from recent results obtained in the use of diphtheria antitoxin intravenously, it would seem that this method should be the method of choice, since the antitodal effects are secured so much more promptly.

REFERENCES

REFERENCES

20. ——— and Reichert, E. T. Smithsonian Contributions to Knowledge, 1890.
CHAPTER XXXV

THE BIOLOGICAL METHODS OF TREATMENT IN CANCER

RICHARD WEIL

INTRODUCTORY

Under the biological methods of treating cancer and other types of new growth are included all those forms of therapy which are based upon immunological principles. During the past ten years there has been an extraordinary and quite unprecedented degree of activity in attacking cancer upon these lines. In part this has been due to the noticeable renewal of medical interest in cancer. In great part it can be traced directly to the stimulus derived from animal experimentation in cancer. The fact that immunological methods have been found to produce certain definite results, even though not of a curative nature, in experimental tumor has naturally awakened the hope that similar procedures might prove effective in human therapeutics. In general, it may safely be claimed that this movement represents a line of advance which is bound to undergo further development in the future. The physical methods, such as radium, mesothorium, or the X-ray, which have recently aroused much enthusiasm, are necessarily limited in their scope and applicability to accessible neoplasms. It is reasonable to demand for cancer a mode of attack which shall be of such a character as to prove effective against all foci of the disease in the body, whether they appear as superficial manifestations or as deep-seated, unsuspected metastases. Treatment of this sort is perforce constitutional, and, according to the nature of the procedure, may be classed as either chemotherapeutic or immunological. The chemotherapeutic method has been more or less systematically tested for centuries, but has hitherto

1 It has become customary in the literature to use the word "cancer" in a generic sense, as inclusive of all types of new growth, although the malignant types are usually especially indicated. Thus the official organ of the International Association for the Study of Oncology is called "Cancer." In the present article the same usage has been followed, and, except where specifically indicated, the term cancer is used in a broad sense, intending new growths in general. The term "carcinoma," however, is generally employed to designate a particular type of neoplasm, with certain characteristic histological and biological properties, and is, therefore, the equivalent of "cancer" used in the narrower, technical sense.
failed to achieve any success. The recent experiments of Wassermann with selenium compounds offer only a vague prospect that the near future will bring much help from synthetic chemical products. The immediate hopes and activities of those interested in cancer therapy will probably in large part, therefore, center in the biological methods of treatment.

These methods have been advocated in very considerable variety of detail and of technique, but in the last resort are reducible to either one of two types, namely, active immunization, or so-called vaccination, and passive immunization, generally described as serum treatment. Each of these principles has been applied with a certain degree of success in animal cancer, and, therefore, has ample warrant for a thorough trial in human cancer. Before discussing the methods and the results which have been described in human disease, it will be advisable to consider the special features which characterize the immunological problem as applied to cancer, and, furthermore, to analyze rather carefully the results which have been obtained in animals. It will be found that there are certain important features which differentiate the situation in the clinic from that which obtains in the laboratory. Furthermore, it will become evident that the immunological problem is essentially distinct from that which exists in the infectious diseases, and that it must be approached with a special technique and, if possible, with finer and more precise methods. It is the failure to recognize these data which renders much of the work hitherto recorded more or less ill judged and ineffectual.

General critiques of these methods of therapy are given in Woglom's (136) review of cancer, and in a recent paper by Klempner and Meidner (74). A critical analysis of the entire situation is to be found in a paper by Ewing, entitled "The Treatment of Cancer on Biological Principles" (53).

The present article will present, first, an analysis of those data of general immunology and of experimental cancer which bear on the therapeutic problem. This section will be followed by a critique of the therapeutic criteria in human cancer and a study of the results which have been obtained in human beings. An intermediate section will be devoted to a brief review of the relationship between the clinical and the experimental data.
practical purposes, it may be assumed that any immune serum which may
be used in human beings will have to be derived from an animal of a differ-
ent species. The problem is, therefore, essentially one in heterologous pas-
sive immunization. The procedure depends upon the correctness of two
fundamental assumptions, namely:

(1) That it is possible, by means of the injection of cancerous tissue
or of related material, to produce a serum containing specific, cytolytic,
immune bodies against cancer cells.

(2) That it is possible, by means of the introduction of such an im-
mune serum, to destroy the cancer cells in the body of the host or patient.

The correctness of both of these assumptions is essential to the success
of the therapeutic method under discussion.

As regards the first assumption, there is, of course, no question that
it is perfectly possible to produce immune bodies by means of the injection
of the cells of one species into an individual of another species. For the
purpose of cancer therapy, however, such a result is inadequate. In order
that the immune serum may not be bound by the other cells or by the fluids
of the cancer-bearing individual into whom it is to be injected, it is
requisite, in the first place, that it should be highly specific. If, for exam-
ple, a rabbit is highly immunized against mouse kidney by means of a
series of injections thereof, the rabbit develops a serum which is found to
be highly destructive of the red blood cells of the mouse, and which also
reacts with the fluid portion of the mouse's blood to produce precipitation.
Moreover, when this same serum is tested against extracts made from vari-
ous other organs of the mouse, as, for example, the liver or pancreas, it is
found that the combination is effective in inducing complement-fixation.
Thus such a serum is in high degree non-specific. If it were introduced
into the body of a mouse with the object of acting specifically upon the kid-
ney of the animal, that object would, in great measure, be defeated. A
considerable part of the immune substances in the serum would be fixed
or bound by the blood or by the organs of the mouse, leaving only a small
residue to react with the kidney. Moreover, the mouse would almost cer-
tainly be killed by the resultant effects upon the blood, such as intravascu-
lar agglutination or hemolysis, long before the specific effects of kidney
destruction could become manifest. It is, therefore, quite clear that a
prime object in the production of an immune serum against cancer must
be a high degree of specificity. The question arises whether this requisite
can be met or even approximated. In the earlier days of immunological
research the answer to this question would have been unqualifiedly in the
negative, but within the last few years there has been a great deal of work
which demands a revision of the entire subject.

In addition, it is not sufficient that the antibodies to cancer should
manifest precipitation, agglutination, or complement-fixation. They must
be truly cytolytic and capable of destroying the life of the cell. An under-
standing of the work which has been directed toward these objects is an essential preliminary to the approach of the cancer problem in human beings.

Specificity of Cytolysins.—The specificity of antibodies is one of the corner-stones of modern immunology, and has been made the basis of a number of very important and reliable medical and forensic tests. This specificity, however, is not absolute. Thus one is hardly surprised, as H. Sachs (114) phrases it, to find that all of the various tissues and fluids of an animal react with an antibody prepared against any one of them individually. Blood serum, milk, and urine produce antibodies against the erythrocytes of the same species. Similarly, as has recently been shown by Pearce (71), immune serum prepared by the repeated injection of dog's bile into rabbits displays a certain amount of hemolytic activity and a well-marked agglutinative action on dog erythrocytes. Similar results have been obtained in a large number of experiments by Ranzi (107) and by Wolff-Eisner (137), in which the antibodies produced by the injection of suspensions of kidney cells, of liver cells, or of spleen have been found to be actively hemolytic. On the other hand, the last few years have brought to light a number of very striking manifestations of the reverse phenomenon, in accordance with which it would seem quite justifiable to conclude that certain of the tissues, certain of the cells, and certain of the pathological products of the animal body possesses a degree of antigenic specificity previously entirely unsuspected. The most important contributions to this subject have been made by Uhlenhuth, Wells, and Dunbar, and later work has been largely along the line of expansion and development of their findings. These contributions to the problem of specificity in immunity are based to some extent on a study of the test-tube reactions, such as agglutination, lysis, or complement-fixation, but in still greater part upon the vital reaction of anaphylaxis. In all of these reactions the active agents are antibodies, so called; but whether the various properties thus analyzed are merely various functions of the same underlying substance, as is held by some authorities, or actually characteristic modes of action of different substances, is not yet known. In the following discussion, at all events, the different reactions are considered to be of equal value in the analysis of specificity, and this is a standpoint which is amply justified by the fact that they coincide very generally in their results when used to control one another.

Specificity of Unaltered Tissues.—In studying the various constituents which could be separated from avian eggs by chemical procedures Wells (131) found that they manifested a very striking degree of specificity when tested by the anaphylactic reaction. Ovomucoid, ovalbumin, and ovovitellin could be very sharply distinguished, even though derived from the same species of egg. Dunbar (43, 44) showed that the pollens derived from various plants produced antibodies which gave a sharp reac-
tion of complement-fixation with the pollen itself, but with no other portions of the plant. He studied the sexual secretion of male and female fish by the same method, and found here, too, that antibody production was very highly specific, inasmuch as the other tissues of the fish failed to react. These findings have been recently confirmed with the anaphylactic method by Kodama (75). Uhlenhuth (125), Uhlenhuth and Häendel (126), and Andrejew (3) showed that the crystalline lens of the eye produces antibodies which are very highly individualized, inasmuch as they fail to give either the reaction of precipitation, of complement-fixation, or of anaphylaxis when tested against other tissues or fluids of the body. On the other hand, these antibodies do react with the lens albumin of other species of animals exactly as Wells had found to be the case for the various identical chemical constituents of the eggs of different species of birds, and as Dunbar had found, though in less measure, of the sperm of different species of fish. The results obtained by these investigators have never been seriously shaken, and they permit of a number of important conclusions. In the first place, it is clear that certain tissues of an organism, whether plant or animal, are highly differentiated in their antigenic properties from all the other tissues of the body. The tissues of which this has been shown are characteristically those which are free from the mechanical admixture of blood. Second, by chemical means it is possible to separate from the cells certain constituents in a pure state, which, again, manifest the same type of antigenic specificity. It seems likely that the chemical measures have served to secure the same degree of separation from all the other cellular constituents which is spontaneously provided by anatomical conditions in the case of lens substance and of spermatic fluid. Thus the observations of Wells furnish the logical point of departure for the chemical separation of a highly specific antigen from the various organs, or from cancer. Third, it is evident that in all of these instances the specificity which is so striking a characteristic of these particular immune sera, when tested against the other organs of the same species of animal, largely disappears when the same tissues or chemical homologs derived from different species are used as antigen.

Thus arises the conception of tissue specificity (Organ-spezifität) as contrasted with that of somatic specificity (Art-spezifität), or species specificity, of the German literature. This latter conception again has been fruitful in various lines of cancer research, as, for example, the miostagmin reaction of Aseoli and the complement-fixation reaction of von Dungern, in which cancer derived either from man or from animals serves equally well as antigen. But, aside from this, it furnishes the rational basis for immunization by heterogenetic cancer in the case of human disease (Gaylord).

In addition to the examples of specificity above cited, it has been shown by Krusius that various products of epithelial activity, such as the hair, hoofs, and horns, which he compares biologically to lens substance, also manifest tissue specificity. Aside from these instances, however, there is practically no type of
tissue or cell of the animal body of which this degree of specificity holds true. It has, indeed, been repeatedly claimed for the choroid and the cornea, for example, only to be subsequently disproven. Recently, however, a fairly good case for muscle specificity has been made out by Stoicesco (122).

Of somewhat special interest in connection with cancer is the observation of Raubitschek (108), who showed that a pathological process, namely, amyloidosis, may endow the tissue so altered with a high degree of antigenic specificity. It must be remembered, however, that this is a pathological product itself highly specialized, and in so far comparable with such other products as the crystalline lens or the hoof. Any inference as to specificity in other pathological conditions, such as tumors, not characterized by the presence of a specific by-product, seems, therefore, very questionable.

Finally it has been shown that a certain tissue derived from the fetus, namely, the placenta, may, upon injection into animals of a different species, give rise to antibodies which do not react with the maternal tissues or fluids. Such an immune substance has recently been obtained by injecting rabbits with blood-free human placenta, and is called "Synechioprecipitin" (72). The specificity so demonstrated is analogous to that illustrated by Abderhalden's reaction for pregnancy, which will be subsequently considered. Its bearing upon the cancer problem is questionable, in view of the fact that the antigen is supplied by a foreign organism, namely the fetus.

There are a few exceptions to the general conditions above described. For example, the specificity of spermatic fluid is not universal. Thus Moxter (98) found that immune serum made against the spermatozoa of the sheep also possessed hemolytic properties. Metchnikoff (93, 94), however, has questioned this result. On the other hand, the lack of antigenic differentiation as between the various tissues of the same animal is also subject to occasional exception. Thus the remarkable series of researches carried on by Forsmann and his pupils (60), and amply confirmed by other investigators, have established the fact that rabbits immunized against suspensions of guinea-pig organs yield sera which are very poor, or entirely wanting, in hemolysins for guinea-pig red cells, while they are rich in hemolysins for sheep cells. These findings are, however, at the present time almost solitary examples of this phenomenon, and it is not at all likely that they represent a widespread condition.

**Summary.**—The net result of all this study has been to indicate very clearly that, with the exception of certain tissues which are anatomically in a special class, the injection of any tissue of the animal body into an individual of a different species gives rise almost universally to the production of antibodies which are not specific for the tissue employed, but react with many of the other tissues, notably with the red blood cells and the serum of the species which supplied the antigen.

**Modifying Factors.**—There are two factors which to some extent modify the above conclusion, namely: (1) the factor of relative specificity, and (2) the factor of relative avidity.

As regards the factor of relative specificity, it is of considerable theoretical importance, but, unfortunately, of slight practical value. Inasmuch, however, as it has been utilized in therapeutic technique, it becomes necessary to give it brief consideration. The essential feature is simply this, that the specific antigen absorbs antibody from an immune serum
more completely and effectively than does any other antigen. Thus, if a rabbit be immunized against goat’s erythrocytes, it will develop a serum capable of producing a considerable hemolysis, not only of goat’s, but also of cow’s red cells. If such a serum be treated under special conditions with cow’s cells, it is not found possible to remove all the hemolysins for goat’s cells, but goat’s cells do effectively remove all the hemolysins for cow’s erythrocytes. On this principle, it has been suggested that sera prepared against cancer might be deprived of their toxicity for the remainder of the organism by previous treatment with red blood cells. It will subsequently be shown, however, that this method of preparation does not, as an actual fact, materially diminish the general toxicity of such a serum.

The second factor has been studied in great detail by Morgenroth and Rosenthal (96, 97). It depends upon the observation that, when a number of antigens are exposed to the action of an immune serum, the avidity of the specific antigen for immune body to a great extent prevents the non-specific antigens from combining with it. It may even be shown that the specific antigen can remove antibody which has been bound by non-specific antigen and appropriate it to itself. Upon this principle, v. Dungern (45) expressed the hope that the use of an anti-epithelial serum might be made practically effective in cancer. He was led to this view by the observation that the presence of epithelial cells in test-tube experiments protected red blood cells from the lytic action of such a serum. Further experiment upon the living animal, however, has shown that such immune sera do, as an actual fact, exert, not a specific, but a generalized, effect, involving especially the cells of the blood.

Therefore, it may safely be said that these two additional factors may, indeed, somewhat modify and refine the conception of specificity, but do not, for the practical purpose of this discussion, materially alter it.

Specificity of Nucleoproteins.—A further advance in the attempt to induce the production of specific cellular antibodies has been made along the lines of chemical differentiation. Although experiments have been conducted with various chemical fractions of the cell, the most interesting results have been obtained with nucleoprotein. The first observers to claim a high degree of specificity for antibodies produced by the injection of organ nucleoproteins were Bierry and Pettit in 1904 (22). They claimed to be able to differentiate between kidney and liver by means of such antibodies, and also to produce typical and isolated organ lesions by the injection of the immune sera. This work has been developed and carried very much farther in a series of publications by Beebe, which extend over the years 1905 to 1910 (14-17). In the first place, Beebe states that he “encountered considerable difficulty in the failure of a large percentage of the animals to produce a highly active serum. For instance, out of a lot of five rabbits inoculated with liver nucleoproteins only one produced a highly active serum.” Beebe prepared antisera against the nucleoproteins isolated
from the liver, thyroid, pancreas, and kidney of the dog. The activity of these different sera was tested for precipitins and agglutinins in vitro against solutions and suspensions of the cellular portion of the various organs of the dog. Although the limits of the reactions are not given, it is evident that they could be produced only with low dilutions of the sera. On the other hand, Beebe states that they were relatively specific, "indicating a decided preference of the serum to combine with the specific nucleoprotein." Agglutinative and hemolytic properties were present only in high concentration, and could be removed by saturation with red cells, leaving the specific "precipitin and agglutinin reactions but slightly diminished." These claims of Beebe have received confirmation from the experiments of Shaffer, although other observers, notably Pearce and his co-workers (102), have failed to determine any specificity in antisera made against nucleoproteins. It should be stated, however, that Pearce prepared his nucleoproteins by a method different from that used by Beebe.

Wells (132) has recently given a critical survey of all the published work upon nucleoproteins as antigens, from which he concludes that "chemical considerations and experimental observations are at present opposed to the assumption that so-called nucleoproteins possess any greater antigenic specificity than do the native cell proteins themselves." In part these divergent results are due to the fact that it is difficult to isolate the nucleoproteins and that some experimenters have accomplished this less perfectly than others, as is evident from the contradictory observations with the anaphylactic reaction. The results of intravital injections will be subsequently considered, but of the test-tube reactions it must at present be admitted that the question of the degree of specificity obtained by injections of nucleoprotein, as compared with the total protein of the cell, is not definitely determined.

SUMMARY.—In conclusion, it may be said that, with the exception of certain very special tissues, at the best only a very moderate degree of specificity can be claimed for the antibodies produced against the cells of the body or their chemical derivatives.

Cytolytic Properties of Antisera.—It still remains to inquire whether the antisera so produced possess true cytolytic properties. This question is entirely independent of that previously considered, inasmuch as it is well known that not all antibodies are destructive of life. Thus the typhoid bacillus may grow freely in a serum which possesses marked agglutinative capacity. This question has fortunately received a fairly reliable answer in connection with the cytolysins. It has been possible to study the effect of immune sera upon cells removed from the body in the case of ciliated tracheal epithelium and of the spermatozoa. Von Dungern (45) injected the tracheal epithelium of the ox into rabbits, and was able to show that the resulting antiserum was capable of paralyzing the ciliary action of these cells. The cells, however, were not actually destroyed or broken down by
the serum. On the other hand, the red blood cells of the ox were hemolyzed by the same serum. It was therefore hemolytic, but not epitheliolytic, in spite of the fact that epithelium supplied the antigen. It is not possible to decide, in this case, whether the hemolysin was an immune body separate and distinct from the anti-epithelium immune body, or whether its presence was not due simply to the admixture of red cells in the immunizing injections. Metchnikoff (94) and Metalnikoff (92) have very emphatically defended the latter opinion in connection with the hemolysins developed by the injection of testicular tissue, and it is certainly to be considered as a possibility in v. Dungern's experiment. Thus we can only say that v. Dungern failed to produce an immune substance capable of dissolving the ciliated cell, although it paralyzed the ciliary activity.

Landsteiner (79) studied the effect of immune serum upon spermatozoa. He prepared guinea-pigs by frequent injections of large amounts of bull's spermatic fluid. When fresh spermatozoa of the bull were introduced into the peritoneal cavity of these guinea-pigs and at the same time into normal control guinea-pigs, it was found that they had completely lost their motility in the immunized animals at a time when they were still active and lively in the controls. Landsteiner makes no statement which would permit one to conclude that the paralyzed spermatozoa were also killed.

The same problem has been studied, under Metchnikoff's direction, by Metalnikoff (92), who states that the serum of a rabbit immunized against the spermatozoa of guinea-pigs "kills" the latter within 3 to 4 minutes. Metalnikoff evidently concluded that the spermatozoa had been killed from the fact that they had lost their motility; this is indicated by a statement of Metchnikoff's in another paper (93) to the effect that he "had never, even with the most powerful spermotoxic sera, observed either a partial or a complete dissolution of the spermatozoa." So that here again there is evidence that the spermotoxin paralyzes the cell, but not that it kills it.

The foregoing experiments demonstrate that the serums prepared against spermatozoa and against ciliated epithelium are capable of exercising a very powerful effect upon the functional activity of these cells. It is perhaps unreasonable to expect the same evidence of cytolytic effects in the case of the other cells of the body as is obtained with the red cells. The latter may be completely dissolved and destroyed by hemolytic serums. It may well be, however, that the paralysis of their motor activities is a phenomenon indicative of the death of the ciliated cells and of the spermatozoa, even in the absence of any marked morphological changes. At all events it has been proven that these cellular antisera effectively inhibit the activities of their antigenic cells.

At this point we come upon what is to be regarded from the standpoint of this discussion as the most important aspect of the problem, namely, the cytolytic effects of antitumor sera upon tumor cells. Very fortunately,
this particular problem has been approached by an entirely new method, that of cultivation of the cells in vitro. Working with this method, Lambert and Hanes (77) have discovered certain facts which have recently been summarized by Lambert (76) in the following words: "Guinea-pigs were immunized by suitable injections against rat tissues, and their plasma used for cultures of rat cells. In the culture preparations made with this cytotoxic plasma there was complete inhibition of growth with partial or complete disintegration of the rat sarcoma fragments." Thus, by means of cultivation in vitro, it can be shown that cellular antibodies, specifically those prepared against rat sarcoma, do actually kill and disintegrate the cell. Of course, it must not be forgotten that the conditions of the experiment permit of a prolonged exposure of the cells to a concentrated serum, a condition which cannot even distantly be approximated by the vital experiment. Lambert's experiments are of further interest as demonstrating that this true cytolytic activity is not specific. He states that "two series of guinea-pigs were immunized, respectively, with rat sarcoma and rat embryo skin. Later plasma from each series of animals was used for cultures containing both kinds of tissue. These experiments showed that sarcoma may immunize completely against skin and vice versa. There was no evidence of even a relative specificity." Red blood cells also were found to immunize against other tissues.

Thus the method of tissue culture has demonstrated not only the weakly cytolytic property of anticell sera, but also their entire lack of specificity.

**Intravital Action of Organ Antisera.** —The importance of the foregoing data will be evident in the discussion of the action of organ antisera when injected into the living animal. Experiments of this nature have been made in very great number and against almost every type of organ, so that it will be possible in this discussion to consider only those which are most instructive from the standpoint of cancer therapy. The sera which offer the greatest interest in this connection are those which have been prepared against the lens, the gastric mucosa, the nervous system, the kidney, and the liver. An antiserum to the ocular lens was prepared by Pick (104) by injecting the lens substance of guinea-pigs into rabbits. This antiserum was injected into guinea-pigs intravenously in the maximal sublethal amounts, and intraperitoneally in large multiples of those quantities, and during the succeeding week their eyes were frequently and carefully examined. "It was in no instance possible to discover any pathological change; in particular, no opacities of the lens could be detected." Antiserum prepared against the lens of various other animals were also without effect in the guinea-pig eye when given in the manner above described. In view of the fact that the lens substance is probably the most highly differentiated tissue, regarded as an antigen, in the entire body, it becomes of interest to inquire into the possible causes of this absence of specific action on the part of the serum. Pick states that the various organs of the guinea-
pig fix the antibodies present in the antilens serum, consequently it is not surprising that this same serum, when given intravenously, may kill a guinea-pig in amounts not greater than one-half cubic centimeter. Thus it seems permissible to conclude that the lens is not affected by the serum for the reason that the other tissues of the body appropriate the antibodies before they can reach the specific antigen, the lens.

In view of the fact that the various immune reactions have demonstrated a greater degree of specificity for the lens substance than for any other tissue, the results obtained by Pick would hardly lead one to expect specific effects for any of the other tissue antisera. And such indeed has been the experience of the majority of observers. Pearce (100) studied the antigenic properties of various organs of the dog after first thoroughly washing the blood out of the vessels of the animal. All sera resulting from the injection of these blood-free emulsions of liver, adrenal, pancreas, and kidney into the rabbit had the power of agglutinating dog’s erythrocytes powerfully and quickly, and also of hemolyzing them. When injected into the living animal all of these sera, except the nephrotoxin, produced generalized lesions. The pancreatic and adrenal sera effected no changes in these organs, but caused degeneration of the liver and kidney. The hepatotoxin produced lesions chiefly in the kidney, with occasional instances of focal necrosis in the liver.

The specificity of nephrotoxin was first maintained by Lindemann (88), and has been confirmed by Pearce. The latter author did, indeed, find an accompanying hemolysin in the rabbit’s antiserum, but even after this had been removed by saturating with erythrocytes in the cold the serum was still powerfully nephrotoxic. The microscopic lesions of the kidney are widespread and intense, and albuminuria is marked and prolonged. “With the exception of the liver, which in some animals exhibited extensive granular degeneration, no other organs appear to be affected by this serum.” Woltmann (138) has confirmed these results. The relative specificity of nephrotoxin is, unfortunately, not of very great theoretical importance, for the reason that practically all the other antisera also produce marked effect upon the kidney. The same objection vitiates the very striking observations of Bolton (27) on the production of gastric ulcer by a serum prepared against the gastric mucosa. Other antisera have the same effect, owing to the acidity of the gastric juice, which digests the partially injured cells. Neurotoxic serum has been prepared and studied by Armand Delille (4) and several other observers. When injected into the brain it produces characteristic and striking symptoms, which never accompany the injection of normal alien serum. When injected into the veins the cerebral symptoms fail to occur, owing to the fact that the antibodies are bound by the other tissues.

It is not necessary to consider in detail the great variety of sera which have been prepared against the prostate, the placenta, the intestinal mu-
cous, and other tissues. In no instance has specificity been satisfactorily demonstrated.

Of sera prepared against the nucleoprotein of various organs, notably the liver and the kidney, it has been claimed by Beebe (14-17) that they produce lesions which are relatively specific. The conditions described by him are certainly very striking, but have been disputed by others, notably Pearce (101). At the present moment, in view of these divergent opinions, no definite judgment is possible on this point.

Beebe prepared his nucleoproteins by precipitation with acetic acid, whereas Pearce raised the hashed organs to the boiling point before they were submitted to extraction. It is possible that the difference in results is partly attributable to the differences in preparation. On the other hand, the sera prepared by Pearce are stated by him to have been more highly specific than those used by Beebe, in view of the fact that they evinced much less side action upon the red blood cells in test tube experiments.

The effects of chemical treatment upon the antigenic properties of proteins have been studied by Landsteiner and Prasek (80). It appears that protein acted upon by heat, acid, alkali, iodin and a variety of other agents produces an antisemum which has an altered affinity for the corresponding native protein. This fact, therefore, introduces a new feature which materially affects the validity of chemical procedures in preparing antigens to the living organs.

Thus all organ and tissue antisera, even those theoretically of high specificity, produce generalized lesions when injected in vivo.

Intravital Action of Cancer Antisera in the Lower Animals.—In conclusion it must be admitted that the vast amount of labor expended on the cytolytic sera has failed to leave much ground on which to base a therapeutic procedure for cancer. It has been possible to produce sera which in the test tube proved to be highly specific, e. g., the antilen serum; but the experiment on the living animal has shown that they possess generalized affinities which rob them of all specific action in the living body. On the other hand, it appears that certain antisera, notably nephrotoxin, have a somewhat special action, with a tendency to produce aggravated lesions in the antigenic organ. Finally it is clear from the work of Bolton in gastrototoxins that certain tissues have marked predisposition to manifest distinctive lesions upon the exhibition of antisera that have a generalized action. This is due to the presence of accidental factors, such as the auxiliary action of the gastric juice, or again, of the excretory function of the kidney. When these data are applied to the cancer problem, it must at once be evident that the prospect for a specific heterogenetic anticancer serum is discouraging. In the first place, as regards the specificity of anticancer sera, it seems quite evident that this represents an unattainable ideal. The majority of those who have produced sera for therapeutic purposes in cancer have not undertaken to make thoroughgoing tests of their specificity. However, Pick (104) has made the observation that
"the guinea-pig lens absorbs specifically the antibodies of a rabbit antiserum against mouse carcinoma." This being the case, it may be assumed with certainty that other tissues would act in similar fashion. In the absence of specificity the only hope for an effective anticancer serum would rest on the presence of some special factor in new growths which might make them particularly susceptible to the action of such a serum. Such a factor, which would be comparable to the action of the gastric juice in Bolton's experiments, might be presented by the increased food avidity of cancer cells, or by their lowered resistance to toxic agents. Whether or not any specific action has been demonstrated will appear from the analysis of reports hitherto made on the action of such sera.

The records of attempts to influence the growth of animal tumors by means of heterogenetic antisera are now quite numerous and complete. Jensen (68), who practically inaugurated the experimental era in animal tumors, was the first to make trial of this method. He injected rabbits with cellular emulsions made from mouse carcinoma, and then reinjected the antiserum into tumor-bearing mice. In a number of cases this mode of treatment was accompanied by the disappearance of the tumor masses. In a subsequent paper (69) Jensen criticised his previous findings on the score that a certain number of his apparent cures might have been attributable to spontaneous retrogression, but he still maintained that the disappearance of the large tumors was not to be explained upon that basis, and he continued to regard them as true cures. His views have not been borne out by the findings of other observers. Ehrlich (50) prepared an antiserum to mouse cancer in rabbits, and submitted the tumor material to its action previous to inoculation into fresh mice; no effect was observed. Many other workers, notably Borrel (28), Bridré (31), and Lewin (82), have prepared antisera by injecting rat or mouse tumor into such different species as sheep, chicken, rabbit, and guinea-pig, and have then tested the effectiveness of these sera upon the vitality of the tumor cells, both in the test tube and in the living animal.

In no instance has it been possible to determine that heterologous antisera prepared against animal tumors have a marked cytolytic effect in vitro, or a curative action in vivo.

An apparent exception to the above conclusions is found in the statement of v. Leyden and Blumenthal (87) that a dog cancer was cured by means of injections of an antiserum prepared in rabbits. The fact that this observation now stands practically alone deprives it of any great significance. Moreover, the microscopic evidence as to the character of the tumor was inadequate, having been made on the aspirated cells.

**Active Immunization**

The second method of biological therapy in cancer is that of active immunization or vaccination.
The theory of vaccination in cancer depends upon two principles, which are very similar to those discussed under passive sensitization. In the first place it is necessary to assume that the animal body can produce antibodies against its own tissues or the tissues of others of its species, and that these antibodies have cytoytic properties; in the second place, that these antibodies are effective in the body of the producer against its own antigenic cells. The validity of the first of these postulates is now quite adequately established, while that of the second is still very questionable. The most striking illustration of their action in the living animal is afforded by the study of experimental tumors. It will be necessary, therefore, to make a brief preliminary analysis of these experimental observations before passing on to the discussion of the therapeutic problem in human beings.

Vaccination in Experimental Tumors

When a favorable tumor derived from another animal of the same species is experimentally inoculated into mice or rats, a certain proportion of the plants "take," that is to say, develop into palpable tumor masses. Of these "takes" some remain as tumor masses during the remainder of the life of the animal and continue to grow more or less actively, while others gradually diminish in size and eventually disappear. The biological condition of the latter animals, as regards their relation to further inoculations of tumor, is found to have undergone a striking alteration. It was discovered by the Buffalo School of Cancer Research, Gaylord, Clowes, and Baeslack (34, 36), that animals which had thus recovered were refractory to subsequent implantations of tumor tissue. To use the terminology of the analogous condition seen in infectious diseases, they had become actively immunized against tumors. This observation has been frequently confirmed, and is now firmly established. Ehrlich (48-50) attempted to show that the immunity conferred upon an animal by the retrogression of an implanted tumor protected that animal against every other histological type of tumor which might occur in the species ("panimmunity"). Bashford and his school, however, have shown that the immunity is relatively, though not absolutely, specific for the original type of tumor (10, 12, 13).

A further advance was initiated through the discovery made by the Buffalo School (35) that animals could be very effectively immunized against tumor implantation by means of preliminary injections of tumor tissue of such low virulence as not to produce any tumor growth. Then, again, the subcutaneous injection of dilute suspensions of cancer cells in small amounts renders an animal resistant to subsequent tumor implantations, although no actual growth has occurred. It has further been shown that not only tumor tissue, but many other types of tissue, can be utilized in the same manner to produce immunity. This comprises not only the cells of the various organs, but even the blood cells. The tissues of the
same species are very much more effective in this regard than are those of other even closely related species, and of its own tissues especially active are the spleen and the skin of embryos of the same species. When the tissues of the same individual animal are used they are much less effective than are those of other individuals; in fact, according to Woglon (135, 136), they are probably entirely ineffective.

Many important experiments have been performed with the object of determining whether tissues which have been altered by various procedures can be used in vaccinating against cancer growth. This is a question of great importance, inasmuch as its decision has been taken by some to indicate the proper mode of therapeutic procedure in human beings. The evidence is now strongly in favor of the view that immunity can be induced only by living cells, whether these are derived from tumors or from normal tissues. Michaelis (95) found that chloroform, Lewin (82) that heat, Haaland (63) that freezing and mechanical disintegration, and Clowes (35) that chemical action destroyed the capacity of the cells to induce immunity toward tumors. Recently Woglon (137) has gone a step further, and has succeeded in showing that the curves of immunity—the rate of increase, the maximum, and the decline—correspond very closely when mice are given preliminary inoculations either with spontaneous tumor, kidney, or embryo skin. As Bashford (9) says, "The immunizing faculty cannot be divorced from the vital activity of the cells," and this justifies the view originally propounded by Clowes (35) that actual cell growth must take place in order that an animal may develop immunity to tumor implantation.

These important deductions would seem to indicate that living tissue must necessarily be used to immunize against cancer. Now it will subsequently be shown that the use of living tissue is practically excluded in human beings, owing to the fact that there are now on record a number of cases in which this method has resulted in the reimplantation of fresh tumor processes. The new school of workers, therefore, has resorted to the use of tumor tissue which has been slightly altered by treatment, in such fashion that it is rendered incapable of growth, but is still, hypothetically, capable of inducing immunity. The question arises whether there is any basis in animal experimentation for this belief. As an actual fact, C. Lewin (88), in a careful study, has shown that the injection of tumor tissue which has been aseptically autolyzed for 1 to 3 days produces a marked effect upon inoculated tumors in rats. Such injections do not, indeed, produce an immunity to subsequent inoculation of tumors. They do, however, produce marked changes in the majority of the growing tumors, including arrest of growth, diminution in size, and in many cases complete recovery from tumors of very large size. It is of course understood that the normal controls do not present any comparable change. Moreover, Lewin found that the autolysate derived from its own tumor strain was
very much more effective than that derived from an alien strain. Blumen-
thal (24), the originator of the method, has recently asserted that 25 to 30
per cent. of cures in large rat tumors can be obtained by this method. J.
Levin (81) believed that the use of autolyzed rat liver produced a certain
amount of immunity in rats. If these results be accepted, it will become
necessary to modify the above-cited view of Clowes and of Bashford to the
extent that cells devitalized by antiseptic autolysis in the icebox are still
competent to produce retrogressive changes in certain strains of rat sar-
coma. This, of course, would give ample warrant to the employment of
the method in human beings.

Fichera (55) maintained that the use of tissues which had been anti-
septically autolysed over two months or more, whether the tissues were
derived from tumors or from embryos of the species, were competent to
induce retrogressive changes in rat sarcoma. This observation he made the
basis for an elaborate method of treatment in human beings, which will be
subsequently described. The results of this mode of vaccination in rat
tumors have recently been carefully studied by Uffreduzzi (124), and also
by Caravani (32). Neither of these authors was able to find that Fichera's
autolysates had the least effect either upon the inoculation or the growth of
rat tumors. It may, therefore, be considered as finally settled that
Fichera's method has no adequate basis in experimental cancer.

The foregoing data demonstrate that it is a simple matter to immunize
rats or mice against the subsequent implantation of tumors. On the other
hand, it is a very difficult matter indeed to alter the normal growth, to pro-
duce retrogression, or to increase the percentage of recoveries in a series of
rats in which the inoculated tumor has already begun to grow. As an
actual fact, it has never been possible by means of vaccination to produce
retrogressive changes in implanted tumors, unless the recent claims of
Lewin and Blumenthal constitute an occasional exception to this rule. This
being the case, it is hardly to be expected that the spontaneous tumors of
rats would show any effect from this mode of treatment. An implanted
tumor is, after all, not homogeneous with the rest of the body, but always
remains as a mass of foreign cells derived from another animal. As such,
it is distinctly at a disadvantage as compared with the native cells, and fre-
cently undergoes retrogression and absorption, a fate which spontaneous
tumors do not exhibit, according to Bashford, in as much as one per cent. in
a thousand observed cases.

Thus it is not surprising that the spontaneous tumors of rats have never
shown the least effect from vaccination. The English school have shown
that spontaneous tumors may arise de novo in animals highly immunized
against other strains. Moreover, an animal having a spontaneous tumor
can be highly immunized against foreign tumors, but can never be im-
munized against a reinculation of its own tumor. These facts constitute
the most serious objection to the treatment of human tumors by vaccines.
The cause of this failure in spontaneous tumors will become evident from the study of certain experiments which deal with the antibodies prepared against the tissues of the animal itself or of others of the same species, the so-called iso-antibodies and auto-antibodies.

Iso- and Auto-antibodies

That it is possible to produce antibodies by injecting an animal with the tissues of others of its own species might have been anticipated from the foregoing analysis of tumor experimentation, and has been amply demonstrated by other methods. In the early days Ehrlich and Morgenroth (51) were able to show that hemolysins could be produced by injecting a goat with the dissolved red blood cells of other goats. This work has been confirmed and carried a step further by v. Dungern and Hirschfeld (47), who showed that iso-agglutinins could be produced in dogs only when red cells which were marked by certain biological differences from those of the injected animal were used.

The work of Kapsenberg goes further and establishes the fact that a guinea-pig may be sensitized even against its own lens tissue. In the same way v. Dungern and Hirschfeld (46) were able to sensitize rabbits against their own testicles, as were also Gräfenberg and Thies.

In all of these experiments it was found that the production of antibodies was much more marked when the organs of the other species were used than when those of the same species or of the same animal were used.

Specificity of Iso-Antibodies.—Inasmuch as the production of iso- and auto-antibodies is now established, it is necessary to inquire into the specificity of these substances. Halpern (64) injected dogs intraperitoneally with suspensions of dog organs, namely: kidney, liver, spleen, pancreas, and testicle. He tested the specificity of the antibodies so produced by means of the reaction of complement-fixation. He found that kidney antibodies reacted not only with kidney, but also with liver, and that pancreas antibodies reacted also with kidney. To a limited extent, however, specificity seemed to be present. Rados (106) investigated the same problem with great precision under Uhrbnth's direction and obtained even more striking results. He injected different rabbits with suspensions of rabbit kidney, of rabbit choroid, and of rabbit cornea, and tested the specificity of the sera by means of complement-fixation. It was found that in each of the above experiments the iso-antibodies were non-specific. Kidney antibodies reacted with choroid and cornea, and vice versa. Thus one reaches the conclusions that homologous antibodies are much less active than heterologous, while they manifest the same disturbing absence of specificity, although in lesser degree.

Cytolytic Effects of Iso- and Auto-Antibodies.—The cytolytic effects of iso-antibodies, aside from the iso-hemolysins, which are, of course, in a class by themselves, have been studied by Metznikoff in the case of the iso-
spermotoxins. He found that these antibodies destroyed, or paralyzed, the spermatozoa of other individuals of the same species. The action of auto-antibodies appears not to have been studied outside of the body.

Effects of Auto-Antibodies in Vivo.—The effects of injecting the iso- or auto-antibodies of one animal into another animal of the same species has never been shown to produce specific cytolytic effects. There is, however, a very interesting problem concerned with the failure of these substances to produce the destruction or even the impairment of the antigenic cells in the body of the animal in which they circulate. This is the phenomenon to which Ehrlich gave the picturesque title of the “horror autotoxicus.” Metchnikoff (92) came to the conclusion that the antibodies (autospermotoxins) were actually bound by the antigenic cells, and that the lack of free complement accounted for the absence of cytoly-sis. Adler (2), however, has reinvestigated the problem, and finds that the antibodies are not bound by the spermatozoa. He believes that the endothelia of the blood vessels prevent the passage of the antibodies. In this explanation he is unquestionably wrong. It can be shown by the anaphylactic method that the antibodies in the circulating blood, whether auto- or hetero-immune bodies, are anchored by the cells of the animal. One can only conclude that there is an automatic provision of some sort which protects the cells of an animal against destruction by the immune mechanism of the same individual or of other individuals of the same species. This provision is probably least effective in the case of the red blood cells, and yet even here the careful experiments of Muir (99) showed that, “even if some substance should appear which acted as an immune body, there is a further provision whereby the complement of an animal should produce comparatively little harmful effect.” The fact that autospermotoxic sera are active in high concentration outside the body does not invalidate this theory. Thus, to the other disadvantages already disclosed in auto- and iso-antibodies, is added the inherent lack of cytolytic power.

Auto- and Iso-Antibodies in Experimental Cancer in Vitro.—Many attempts have been made to demonstrate the presence of antibodies in the blood of animals which have recovered from implanted tumors. It has been claimed by Clowes that complement-fixation is produced by such sera, and the results obtained by the Abderhalden reaction have been interpreted in the same sense. If such antibodies are actually present it can be shown that they have very little, if any, active cytolytic value. Bashford (6, 7) obtained doubtful results by submitting the tumor graft to the action of such immune serum previous to inoculation into the body. On the other hand, Lambert and Hanes made use of tissue cultures to see “whether the cells of rat sarcoma would grow in the plasma of a rat in which the sarcoma would not grow when inoculated, that is, an immune rat. It was found that the tumor cells would grow just as well in the
plasma of such animals as in that of susceptible or tumor-bearing animals.” It may here be recalled that the cells of rat sarcoma were destroyed in tissue cultures by the serum of guinea-pigs immunized against them. Thus the distinct inferiority of isolsins, as compared with hetero-
lysins, is borne out by the results of this striking experiment.

**Action of Auto- and Iso-Lysins in Experimental Cancer in Vivo.**—It has already been shown that inoculated tumors, when growth has once begun, are not affected by vaccinating the animal with the tumor tissue. It might be urged that in such animals for some reason antibodies fail to be produced. The action of these antibodies upon growing tumors has, therefore, been approached by other methods. Clowes (35, 36) injected mice bearing actively growing, transplanted tumors with serum derived from recovered mice. He came to the conclusion that a distinct effect could be obtained in this way, inasmuch as the tumors in a certain number of individuals were made to retrogress and disappear. C. Lewin (84) reached similar conclusions in a small series of rat sarcoma. Recently I (130) have repeated these experiments with rat sarcoma, and have uniformly failed to obtain any effect upon the tumor, in spite of the injection of relatively enormous amounts of serum. Rous (112) made use of the method of parabiosis, uniting rats which had been proven to be resistant to the Flexner-Jobling tumor with rats bearing the same strain of inoculated tumor. In no instance, however, was he able to discover any effect upon the growth of the tumor. Thus the treatment of tumors in the living animal with serum derived from immune individuals of the same species fails to give evidence of any definite effect.

In this connection, however, an apparent exception to the rule must be noted. Crile and Beebe (41) found that dogs growing inoculated lymphosarcoma could be cured by transfusion of large amounts of blood from recovered dogs. They also observed, however, that the same result could occasionally be produced by means of transfusion from resistant animals. Moreover, Beebe found that a dog could not be protected against inoculation by the preliminary transfusion of immune serum. The results of these experiments, therefore, cannot be considered conclusive at the present time of the presence of immune bodies in the serum. The transfusion may, as suggested by Ewing, act as a non-specific factor, which upsets the balance between the aggressiveness of the tumor and the resistance of the dog, in favor of the latter.

**Summary.**—It is, therefore, evident from the preceding analysis that rats and mice can be immunized very effectively against the inoculation of tumor derived from other individuals of the same species. This process may be compared to the demonstration that iso-antibodies are easily produced. On the other hand, the tissues of the individual itself do not immunize the animal against tumor implantation. This is to be compared to the fact that auto-antibodies can be produced only with some difficulty and that they show no tendency to react in vivo with the tissues.
of the immunized animals (lens, spermatozoa). It follows, naturally, that an animal cannot be immunized against the reimplantation of its own spontaneous tumor, although an animal with a spontaneous growth can be immunized against implantation with a foreign strain. Thus it is very easy to see that the cure of a spontaneous tumor cannot be accomplished in rats or mice by means of vaccination, either with their own tumor tissue or with tissues of other animals. Such a result has never been accomplished. Indeed, even inoculated tumors, after their growth has once begun, have never been affected by vaccination, unless the recent results described by Blumenthal and by Lewin constitute a rare exception to this generalization. Finally we have the statement of Bashford that tumors in rabbits and in guinea-pigs follow exactly the same laws. The only apparent exception is the statement of v. Leyden and Blumenthal that spontaneous tumors in two dogs were cured by vaccination, and it is perhaps wiser at the present time not to lay too great stress on isolated observations of this sort.

To conclude, experimental cancer research thus far gives practically no hope that human tumors can be affected by means of vaccination as a therapeutic procedure.

THE RELATIONSHIP BETWEEN CLINICAL AND EXPERIMENTAL DATA

It has been shown that clinical cancer offers very little prospect for successful treatment, if the results of animal experimentation be accepted as offering a fair analogy. This conclusion is not absolute. It is, indeed, true that the spontaneous tumors of the lower animals offer a far-reaching and almost perfect analogy with those of human beings. In spite of the views of Hansemann (65), there is no question of this fact. On the other hand, there are certain very striking differences in the biology, and especially in the mechanism of immunity of man, as compared with some of the lower animals, which in some ways materially affect the analogy. Thus the leukocytes in man and in the dog possess a proteolytic activity, as tested on gelatin plates, which is immeasurably greater than is shown by the leukocytes of rabbits or guinea-pigs. The character of the complement in rats and mice, as shown by Ritz (111), differs in a striking way from the complement of guinea-pigs or of man. The anaphylactic reaction, again, which is readily elicited in guinea-pigs and rabbits, and more or less easily in man, can be produced only with great difficulty in rats and mice. These few illustrations will suffice to indicate that one cannot draw an absolute inference from rats to human beings, either as regards the mechanism of immunity, or as the means of defense against cancer. For this reason, while it is necessary to keep carefully in mind the results of experimental cancer research, at the same time the data of clinical therapeutics must be independently weighed on their own merits.
THE CRITERIA OF THERAPEUTIC EFFECTIVENESS IN HUMAN CANCER

Before entering upon a detailed consideration of the results obtained in human beings certain important data must be emphasized which constitute the therapeutic criteria in human cancer. Upon this matter I have found it necessary to dwell at some length in a previous publication (128), and the remarks which I then made are pertinent and applicable to the claims which have been made by various writers in describing the therapeutic results of the vaccine and serum methods.

In determining the effects of any given mode of treatment on a tumor a variety of criteria may be relied on. Circulatory changes in the tumor, the relief of pain, and the restoration of a secondarily impaired function are certain of the criteria on which stress has been laid by the majority of observers in the past. Important as are these criteria in determining the progress of purely inflammatory processes, it is unquestionable that their value in judging of the effects of therapeutic methods when applied to malignant disease is open to criticism. It is a curious and interesting fact that almost every therapeutic claim made in recent years in connection with cancer has included among its virtues the relief of pain. In view of this very general effect, not much stress can be laid on this symptom, and it is probably fair to assume that in the great majority of these cases the result is in no small measure psychic. The improvement of function is also largely a subjective phenomenon, and as such requires most careful criticism. Osler relates that he has known a patient with gastric cancer to be relieved of digestive disturbances and to gain eighteen pounds in weight as the result simply of the visit of a sanguine consultant who denied the presence of a tumor. Improvement in the ability to chew food, to articulate words, or to move a limb are phenomena familiar to those who attempt to treat cases of cancer. The victims of this disease seem to be in a very high degree "suggestible" and impressionable, and respond nobly to every therapeutic effort.

Circulatory changes in tumors offer an interesting group of clinical symptoms. The observation has often been made, especially in ulcerated new growths, that treatment is associated with swelling, peripheral hyperemia, and an altered character of the discharge. In spite of the fact that there is no reasonable relationship between this congeries of symptoms and the actual cure of the tumor, they generally receive considerable emphasis, and are cited as an indication of the specific local action of the agent employed. It is the case, however, that the growth may continue to advance in spite of their presence.

Excluding from consideration all of these secondary factors, we may conclude that the observation of the size of the tumor itself is the sole
criterion on which we can place reliance in judging of the effect of therapeu-
tic measures. This implies, in the first place, that a tumor must be
accessible to fairly accurate measurement. Tumors of the uterus, for
example, and intra-abdominal growths will only exceptionally fall into
this class. In the second place, indirect evidence of a decrease in the size
of tumors, such as is afforded by the increased permeability of obstructed
passages, as in the case of tumors of the esophagus, pylorus, or intestine,
must be accepted only with great reserve. Remissions in the obstructive
symptoms characteristic of such tumors are a frequent feature of the
normal evolution of the clinical history of such growths. The relief of
obstruction, however, may be due either to necrosis of the obstructing por-
tions of the tumor, while the remainder continues to grow progressively,
or to a relief of the accompanying muscular spasm. Finally, evidence of
decrease afforded by the roentenogram is not sufficiently exact in most
cases to afford ground for so important a conclusion as that at present in
question.

Not only must there be unquestionable evidence, however, of the dimin-
nution in size of the tumor, but this diminution must be of a kind not
ordinarily attributable to the natural evolution of the tumor. For ex-
ample, it is stated by one observer that "a case of multiple carcinoma of
the face and neck is markedly improved as a result of the treatment." With-
out venturing any opinion as to the conditions which obtained in this
particular case, it is safe to say that multiple tumors offer enormous diffi-
culties in the matter of interpreting therapeutic results. At present we
have in the wards of the General Memorial Hospital a patient with mul-
tiple metastatic carcinomata of the skin. For several months we have at
intervals made accurate measurements of certain of these tumors, and
have found that some have undergone retrogression, others have entirely
disappeared, while still others have continued to grow steadily. In the
case which afforded the ascitic fluid used in Hodenpyl's experiments many
of the lymphatic metastases underwent complete retrogression, while the
metastatic process in the liver, as was demonstrated at necropsy, increased
progressively, and ultimately almost destroyed that organ. Thus in mul-
tiple carcinosis the retrogression of individual nodules is no indication that
therapeutic intervention has produced an improvement.

I shall not delay to emphasize those variations in the size of solid
tumors which accompany hemorrhage and its absorption, edematous
swelling, necrosis in the depths, and other familiar factors which cli-
cially simulate or induce the softening and the reduction that are so often
attributed to therapeutic interference. But it is important to draw atten-
tion to a similar feature in that type of superficial epithelioma known as
rodent ulcer. These new growths not infrequently advance at one point
of the periphery, while they recede at another, and thus cicatrization and
contracture may simulate a partial recovery. This effect is due in part
to alterations not in the growth itself, but in the accompanying ulcerative process. The secretions from the growths, especially if confined under dressings, may have eroded and destroyed the surrounding skin, and it is tempting to interpret a recession of the associated ulcerative disease as an indication of a favorable effect on the new growth. It is unquestionably this aspect of rodent ulcers which plays so generously into the hands of the numerous nostrum vendors for this disease.

In brief, the demonstrable reduction in size of a tumor, of a kind not to be attributed to the natural processes of evolution of that tumor or of its associated lesions, is the one essential feature of effective therapeutic intervention.

The same attitude has been even more forcibly emphasized by Klemperer (73), the Director of the Cancer Institute of the Charité Hospital, in Berlin, in the following words: "The observation of inoperable tumor cases furnishes so many instances of the prolonged persistence of life, of bizarre variations in the course of disease, of the astonishing subsidence of severe symptoms, that we can draw no inference from supposedly therapeutie effects in individual cases. Above all are the so-called cures of sarcoma cases to be regarded with suspicion, for this disease not infrequently shows a tendency to spontaneous remissions amounting even to complete disappearance of the tumor, which may subsequently be followed by a renewed activity."

Finally much stress has been laid by some writers on the prevention of metastasis, or the delay of recurrence after operation, either of which they are prone to ascribe to the effects of treatment. Statements of this character are so exceptionally misleading that in most instances they can safely be ignored. Every surgeon has had the opportunity to see cases in which an apparently incomplete operation was followed by prolonged or permanent freedom from recurrence. Thus Trotter (123), in his recent Hunterian Lecture on "Malignant Disease of the Mouth and Pharynx," has said the following: "All of us, no doubt, have had patients who have refused to submit to gland operation, and some of whom have remained free from recurrence." Not only is this true, but it is not an infrequent observation in certain types of sarcoma that incomplete removal may be followed by an apparently perfect recovery, without local recurrence.

These facts have been almost persistently ignored by the majority of those who have written on this subject. In the ensuing pages it will be found that many of the so-called therapeutic triumphs which continue to echo down the literature must be thrown out on the basis of the foregoing criteria.
CLINICAL DATA

RESULTS OF SERUM THERAPY IN HUMAN BEINGS

The experimental study of the applicability of serum therapy to the cure of animal tumors, under conditions ideally devised to bring out its value, has absolutely failed to vindicate it. Such a result was almost a foregone conclusion, reasoning from the data which had previously been described in this article. It will now be a comparatively simple matter to consider the claims and contentions which have been made for this method in human therapy. A considerable number of these contributions date from a period in which immunological methods were so imperfect that the results do not justify further consideration. Two papers of more recent date, however, demand more careful attention, namely, those of Bosc (29) and of Vidal. Seven papers of the latter author, bearing upon this subject, cover the years 1905 to 1910, and are summarized in his final report, presented in 1910, before the Second International Conference for the Study of Cancer (127).

Other contributions to the subject have been made by Richet-Hericot (66), Beretta (18), Bourreau (30), Ferré (54), Salviati and De Gaetano (115). They date from a period preceding the development of modern immunology, and will not receive detailed consideration.

Bosc (29) immunized three donkeys and one sheep by injections of human cancerous material. The serum obtained from these animals was given subcutaneously in five cases of malignant disease. Although there were general improvement and a diminution in the volume of the tumors with relief of pain, in no instance was a cure accomplished.

Vidal (127) has unquestionably made the most remarkable and pretentious contribution to this subject, comprising 94 cases, of which 6 were cured and 19 were benefited. His methods are very elaborate and differ from those used by any previous investigator. The sera are prepared by injecting human tumor tissue intravenously into dogs. The tumor tissue should be very fresh, and the tumor cells are saturated with a dog antitumor serum previous to injection, and are then freed from this serum by washing. At most three such injections are made. Each of these injections is preceded by the injection either of nucleic acid or of a serum made in rabbits against dog serum, with the object of inducing a preliminary leukocytosis. The serum produced by these measures possesses, according to Vidal, the following properties. The specificity of a serum prepared against human breast cancer was tested according to the method of complement fixation of Bordet and Gengou. The serum gave no evidence of fixation when tested against benign tumors of the breast, such as adenoma, or against normal breast tissue, or against cancers derived from other
organs. On the other hand, fixation was demonstrated against breast cancers derived from other individuals. Conjoined to this specificity was the property of "dissolving" the cancer cell outside of the body. Thus Vidal apparently had at his command a serum possessing all the qualifications necessary to therapeutic success. Before considering the therapeutic results it seems not superfluous to inquire into these preliminary claims for the serum. As regards the method of preparation, in spite of the elaboration of detail, we can only say that it presents no essential alteration of previous technique. Preliminary sensitization of the cells very probably increases the celerity of reaction, and an artificial leukocytosis may possibly enhance its activity, but it is not credible that these measures serve to produce a reaction essentially different from those previously described. Hence it is not possible, in the absence of detailed protocols, to accept Vidal's statements concerning the specificity of his serum.

Another point in Vidal's technique is worthy of special consideration. He discovered that his sera had a marked influence upon tumors in the early stages of treatment, but subsequently lost their effectiveness. He concluded that this might be due to the development, on the part of the patient, of antibodies to the dog's serum, thereby neutralizing its therapeutic value. This theory he verified by test-tube experiments. In order to obviate this unfortunate effect he injected normal dogs with the serum, or rather the pleural effusion, of individuals who had previously received the dog antitumor serum. This serum, described as "N," in the test tube showed the power to neutralize the antdog bodies in human serum, and thus restored the primary cytolytic property of dog antitumor serum in a mixture containing all three of these sera. Very curiously, this antihuman serum of the dog had no cytolytic effect on tumor cells. In order to improve the potency of dog antitumor serum, therefore, Vidal made a practice of injecting with it certain amounts of serum "N." The results were in striking accord with the author's expectations.

These various statements of Vidal's are open to so many grave objections that it is quite impossible to accept his results. It is, however, worthy of note that he has pointed out what is probably the weakest point in the practice of cancer therapy by heterogenous immune serum. It is unquestionable that the injection of any antiserum will rapidly be followed by the development of anti-antibodies on the part of the recipient. I have shown that this reaction may occur as early as three days after the first injection, and that antibodies subsequently introduced into the living animal are thereby destroyed.

Metalnikoff (92) has demonstrated the effectiveness of such anti-antibodies in test-tube experiments upon spermotoxin and antispermotoxin. Hence we may conclude that the effectiveness of heterogenous sera is necessarily limited to the brief period of time preceding the production of antibodies by the patient.
CLINICAL DATA

Vidal's series comprises epithelioma of the tongue, carcinoma of the rectum, cervix cancer, two mammary cancers, and a round-cell sarcoma of the trapezius muscle. All of these received treatment extending over a long period of time, and had remained well from three to ten years. In each case there was microscopic confirmation of the diagnosis.

It is difficult to pass judgment on these claims of Vidal. One can only say that they stand entirely alone and unsupported, and that they are upheld by a mass of very faulty scientific observations.

Berkeley (19) has reported encouraging results in a preliminary paper on the use of a serum prepared against tumor nucleoproteins.

Summary.—This section of cancer therapy may be concluded with the following analysis. The preparation of truly specific antibodies to any tissue of the body excepting lens and germ cells has never been accomplished. Such sera are but very feebly cytoytic in the test tube. Their injection into the living animals produces generalized lesions, involving especially the kidneys. If sublethal doses are employed the injected animal very shortly develops anti-antibodies, which would tend to neutralize any effectiveness which the serum might possess. For all of these reasons it seems certain that no serum prepared or used according to current conceptions has been, or can be, effective against human cancer.

VACCINE THERAPY

The vaccine treatment of human cancer has been practiced in either one of two ways; either the tissue has been used in unaltered form, or it has been previously submitted to autolysis. In addition, it will be necessary to consider the re-injection of the patient's own fluids, which is often described as "autovaccination."

Unaltered Vaccines.—In connection with the use of unaltered cancer elements as vaccines, the fact may be recalled that curative effects have been described in experimental cancer only by v. Leyden and Blumenthal (87) in dog tumors. In mice and rats it is safe to say that the tumors are not affected by this method of treatment. The authors who have published the results of treatment by this method in human beings are Blumenthal and v. Leyden (86), Seeligman (117), Delbet (42), Bertrand (20, 21), Coca and Gilman (38), Rovsing (113), and Coca, Dorrance, and Lebredo (37).

In all v. Leyden reported three cases, which represented the best effects obtained in a considerable series of observations. In the first case an exploratory operation revealed an inoperable mass apparently originating in the region of the pancreas. The woman received subcutaneous injections almost daily over a period of five months, the amounts ranging from one to four cubic centimeters. The material injected was a suspension of a pancreatic cancer removed from another
individual. The tumor became smaller, and was finally no longer palpable by clinical examination; the woman gained twenty-three pounds during treatment; she was kept under observation for a year longer, during which there was no evidence of a recurrence. The second case presented a large pelvic recurrence after hysterectomy for cancer and, in addition, choked disc. The use of vaccines improved vision, while the tumor became smaller. In a third case spinal metastases following upon the removal of a carcinoma mammae were made to disappear, as shown by the X-ray photographs.

In view of the fact that these cases have been selected out of a much larger number as illustrative of the occasional brilliant results obtainable by vaccine therapy one can hardly feel that they justify much enthusiasm. In spite of the great reputation of v. Leyden it must be evident that the diagnosis was not clearly established in Case 1, which may well have been a pancreatic cyst; that the improvement in Case 2 is paralleled by many untreated cases; and that the interpretation of Case 3 is, at the best, doubtful.

Seeligman (117) observed no definite results in uterine cancer.

Delbet passed the tumor removed at operation through a fine grinder, and immediately re-injected the total product subcutaneously. This was done in thirteen cases, and in no instance did it cause either local or general disturbances. In the three cases in which Delbet was able to judge of the therapeutic effect he found it to be nil. In the remaining cases he states that only a prolonged period of observation would permit of any conclusions. He has made no further report on these cases.

Bertrand's (20, 21) case was treated by means of injections of a glycolytic ferment, in addition to the use of cancer vaccine, but it seems unlikely, as Bertrand himself states, that the ferment played any rôle in the outcome. The case was a woman of 45, who came under treatment for a recurrence after amputation of the breast for carcinoma. During the first two months of observation the recurrences grew from pea-sized nodules to exuberant cauliflower growths, and six months later there were numerous scattered nodules in the skin of the chest. During this period of active growth the patient had received subcutaneous injections of very minute amounts, ranging from one to two-tenths of a cubic centimeter of the preparation used. With the injection of larger amounts, ranging up to two cubic centimeters, however, a change became manifest. There was a rapid and progressive diminution in size of the tumors which, within three months, had completely disappeared. The patient was well one year later. The material used by Bertrand consisted of comminuted cancer tissue, dried in a desiccator, and rubbed up with salt solution.

Røsning (113) used tumor material obtained from the patient, which was prepared under Madsen's direction with one half per cent. of carbolic acid. Ten cases of cancer treated in this manner showed no ef-
fects. Of seven cases of sarcoma Rovsing reports three which showed very striking results. A case of sarcoma of the tibia, which had had repeated recurrences after operation, was finally treated by vaccination. Eighteen injections, ranging from one to five cubic centimeters, were given over a period of five weeks. The glandular metastases, as well as the local tumor masses, disappeared, and the case was well at the time of the report, more than a year later. Two other cases, a sarcoma of the soft parts of the thigh and a melanosarcoma of the liver, also underwent apparent recovery. The remaining cases have not been reported on.

Coca and Gilman (38) studied the effects of injecting large amounts of emulsified tumor tissue, repeating the injections at intervals of about two weeks. They treated fourteen cases, two of which presented striking effects.

Case 1 presented a mass measuring 7 by 4 cm. below the angle of the right jaw. Enlarged glands could not be made out, "as the entire side of the upper neck was indurated." The mass was removed as far as possible by operation, and 20 grams of the tumor material were ground up and injected subcutaneously. This was followed a month later by an injection of material derived from a large tumor "probably derived from the epithelial vestiges of the branchial arch," removed from another individual. The masses left after operation disappeared almost entirely during the course of the following two weeks, and the tissues of the neck became normally soft. Unfortunately the patient died of heart disease within eight weeks of his operation. This case is undoubtedly of much interest, but loses in value by virtue of the absence of histological examination of the tumor tissue.

The second case was substantiated by microscopic examination as a carcinoma of the right buccal mucosa, with involvement of the lips, the sublingual and lymphatic glands, and the lymphatic glands of the right side of the neck. After an incomplete removal the tumor tissue was ground up with carbolic acid and injected in two doses, separated by a two-weeks' interval. The wound healed well, induration and ulceration disappeared, and the patient was free from recurrence ten months after operation.

The most thorough and scientific analysis of the results of this mode of vaccine therapy in human cancer has been presented by Coca, Dorrance, and Lebredo (37). They report on 79 cases of malignant new growth, including both carcinoma and sarcoma, in which they re-injected the patients with their own tumor tissue, comminuted in a grinding machine, and sterilized by formalin. Four of these cases bore large tumors, which underwent marked reduction under treatment, amounting in two of the cases to an almost complete disappearance of the malignant tissue. In all of these four cases, however, the therapeutic injections had resulted in a more or less severe streptococccic infection, with fever ranging up to
104° F. The authors are of the opinion that in these cases the favorable action of the injections was due to the intercurrent infection, which, as they say, has been known to produce a favorable effect upon tumor processes. "This assumption," they believe, "is greatly strengthened by the fact that among all the large numbers of cases who received similar injections without becoming infected there were only two in whom the tumors apparently underwent a reduction in size." In the two latter cases they attribute the diminution in size "to the absorption of the inflammatory exudate." In these six most favorable cases the improvement observed was not permanent, and the cases eventually took their natural course.

The authors further point out that the inefficacy of this mode of treatment is still more strikingly manifest from the fact that it failed to prevent early recurrence in the majority of cases where there had been "complete" surgical removal of the growth.

It is worthy of note that general cachexia, local pain, and swelling were materially benefited by this mode of vaccination.

Finally there are reports of more or less ambiguous character by Lunckenbein (90), Werner (134), and others, which do not add any important facts to the discussion.

It is not easy to reach a definite opinion concerning the efficacy of this method of treatment. It seems fair to conclude that certain cases, notably those of Bertrand, Rovaing, and Coca, were strikingly affected by the procedure. Coca's theory that the benefit in three of his cases was due to an intercurrent streptococcus infection does not seem convincing, in view of the fact that the infection was apparently well localized and of short duration. It seems possible, on the other hand, that the action of the vaccine upon the tumor process was enhanced by the injurious effect exercised upon the cells either by the toxins or by the temperature of the infectious process. That the latter factor plays a rôle has been asserted by Vidal, Ewing, and others. Against the very rare cases of apparent cure, and the few cases of striking amelioration, are to be balanced the vast majority of cases in which no real benefit was conferred. The cases in which metastasis and recurrence were prevented may fairly be excluded from consideration on account of the element of uncertainty in the observations. It would be of the greatest importance to determine the factors which make for success in the few cases and those which defeat it in the many. Unfortunately the analysis of the data does not permit of any reliable deductions. The best results seem to have been obtained by the use of considerable doses over long periods of time, the tissue being derived either from the patient's own tumor or from a closely related process in another individual. Comminution of the fresh tissue by means of mechanical grinding, the addition of either formalin, toluol, or carbolic acid to the suspension in salt solution; and preservation in the ice-
box, with or without intermediate sterilization at 56° C., have been the
essential features of the technique. Whether the differences in the final re-
sults have been due to certain variations in technique, either in the prepara-
tion of the material or in its injection, and whether the use of the tumor
taken from the patient himself produces different results from that de-
erved from a different individual, these are questions which only time can
settle. At present it appears as though an occasional tumor, for some
unknown reason, is favorably affected, while the great majority present
no alteration. This, of course, is the most discouraging feature of the
situation, and has militated successfully against the further adoption of
the method. Indeed, its use appears to have been in great part aban-
donned, even by those individuals who at first published favorable reports.
The Heidelberg Institute, from which Werner (133) gave hopeful prog-
nostications, apparently no longer employs the method, and Rovsing has
reported no further cases. This fact constitutes perhaps the most serious
indictment against the method.

**Autolyzed Vaccines.**—Of recent years the use of unaltered tissue as
vaccine material has been largely superseded by the use of autolysates.
A number of factors have contributed to this result. In the first place, a
number of instances have been recorded in which the subcutaneous in-
jection of unaltered tumor tissue has resulted in the implantation of a
fresh tumor growth. This has, indeed, occurred rarely; still its very
possibility constitutes a serious deterrent to the use of that method. Aside
from this, however, the use of autolysates received a marked stimulus
from the experimental and the clinical results described by Fichera. In-
as much as the experimental data have already been criticised, nothing
further need be said here on that side of the subject. At the present time
the use of autolysates is the favorite method in vaccination.

As regards the use of the term "autolysate," there is some confusion
in the literature. Rovsing, for example, speaks of his preparations as
autolysates, and yet there can hardly be any question that they are im-
properly described by that term.

Tissue kept for three days in the icebox in the presence of antiseptics
has certainly undergone no marked degree—one might almost say no
perceptible degree—of autolytic change. On the other hand, there is no
question that Fichera employed highly autolyzed material, and that other
writers have followed the same procedure in less degree. Those who have
used this mode of treatment in one form or other on human beings are
Fichera (55-59), Pinkuss and Kloninger (105), G. Klemperer (73), C.
Lewin (85), Stammler (120), Sellei (118), and v. Graff and Ranzi (62).

The methods of preparation of the vaccine differ, as has been said, in
important particulars. As most typical of mild autolysis may be taken
the description of the procedure followed by Blumenthal. The tumor
tissue is cut into small pieces, and then crushed in a mortar with chloro-
form water. Ten per cent. of chloroform (or toluol) is added to the mass, which is then kept in the incubator for three days at 39° C., being shaken every day. The supernatant fluid, containing flocculi of tissue, is decanted, and injected subcutaneously. The infections are repeated at intervals, usually at a distance from the tumor.

HIGHLY AUTOLYZED MATERIAL.—Fichera prepared his vaccines by autolysis prolonged over three to four months. On the basis of his animal experiments he used autolysates made from human embryos, instead of from tumor tissue. He is of the belief that autolysates, prepared in this fashion, do not act as vaccines, and do not induce immunity. Rather, they supply certain ferments to the organism, the absence of which has led to the disturbance of equilibrium manifested as tumor formation. He prefers to describe his method as "histogenetic chemotherapy."

In 1910 Fichera (56) reported the results obtained in 39 cases of inoperable tumor, in 25 of which treatment was kept up regularly. In seven of this series he observed diminution in the size of the growth. Microscopic examination of the tumor during treatment shows marked cytolysis, a reactive leukocytosis, the presence of numerous macrophages, and a connective tissue proliferation. One year later Fichera (57) reported in further detail on 18 of 36 cases treated. Of these 8 had received no benefit, 5 were improved, and 5 showed a condition interpreted by Fichera as indicative of cure. The criterion of cure, however, is apparently not the complete disappearance of the tumor, but its substitution, as evidenced by the microscopic examination of specimens removed therefrom, by a connective tissue reaction. This is a criterion which would hardly prove acceptable to most clinicians, and which certainly does not justify either the activity with which Fichera has proclaimed it, or the reputation which it has achieved. In his latest papers Fichera (58, 59) gives no further data concerning his human cases.

Babcock (5) has reported upon 21 cases in which he made use of Fichera's method of treatment. He concludes as follows: "We have obtained no evidence that indicates that the use of fetal products has any specific action in the treatment of malignant disease in the human." Lintz (89) also has tested the use of autolysates clinically, and has found no therapeutic effects.

Of Fichera's work one may say, therefore, that it has no solid basis, either in theory, experimentation, or observation.

SLIGHTLY AUTOlyZED MATERIAL.—Pinkuss and Kloninger (105) have prepared autolysates by a variety of procedures, which need not be further described. They treated eight cases, in two of which recurrence after amputation for carcinoma of the breast had been prevented for periods of from four to seven months. Such a claim is, of course, entirely unwarranted.

Lewin (85) observed improvement in a case of carcinoma of the breast
following the use of vaccines. Klemperer and others have described similar unconvincing effects. Graff and Ranzi (62) report on nine cases treated in Eiselberg's clinic with autolysates. In three of these cases, in spite of the fact that the operation was very radical, the prognosis was poor as to recurrence. Under treatment by autolysates the cases have been free from recurrence over periods ranging from two to four years. The authors draw no conclusions. Sellei (118) reports six cases with improvement.

In the entire literature dealing with this branch of the subject there is not a single convincing case, except that of Stammler (120), which was reported from Kümmler's clinic in the Eppendorfer Krankenhaus in Hamburg. This case deserves somewhat fuller consideration. A woman of 65 was operated on in May, 1911, for adenocarcinoma of the uterus (microscopic examination). In six months she had returned with her vagina full of ulcerated masses of tumor and a large metastasis in the groin. She received a curettage of the vagina, followed by acetone. The glandular metastasis was removed and prepared by autolysis for two days at 37°C. in toluol. It was given intravenously in three injections. After six months the case showed marked improvement and was given injections of an autolysate made from a different tumor. By October, 1912, she was apparently cured, and remained so to the date of report (March, 1913). Stammler reports in less detail a more recently observed case of cancer of the lip, in which the results appear to have been very striking.

One may summarize the work hitherto published upon autolysates by the statement that, with the exception of Stammler's case, it presents not a solitary fact of importance. A single observation cannot be allowed to weigh too heavily in the scale in the presence of so overwhelmingly negative an outcome. Moreover, it may be said of Stammler's method that it certainly represents an extremely mild application of the principle of autolysis.

Thus the use of autolysates appears to have little justification either in animal experimentation or in clinical observation. The results obtained with vaccination by means of unaltered, preserved tumor tissue, although discouraging enough, are certainly superior to those obtained with the autolysed material.

**Autovaccination.**—A word is necessary in connection with a method of treatment which was most extensively studied by Hodenpypyl, and which is known in the German literature under the name of "autovaccination."

Mackay, in 1907 (91), described the spontaneous cure of an advanced case of cancer en cuirasse, which accompanied the absorption of a bilateral pleural exudate. Mackay attributed the striking change in the tumor to some effect produced by the absorption of the fluid. Hodenpypyl (67) followed the same idea in employing as a therapeutic agent the ascitic fluid derived from a case of cancer in which there were clinical
indications of high resistance to the advance of the disease. He treated a large number of cases in which the early results were good, but in none of which recovery occurred. A careful study of this fluid, which I made at the time (129), failed to reveal the presence of immune bodies of any kind. It is, therefore, most likely that the method actually represents a species of vaccination by dissolved and partially autolyzed cancer cells.

Lewin (85) reports a very questionable "improvement" in a case of cancer of the breast, with abdominal metastases, treated by this method. It is quite characteristic of the general tendency of the literature on this subject that Blumenthal (25), in referring to this case, describes the result as an astounding success ("verblüffender Erfolg").

**Summary**

In summarizing the results of biological therapeutics in human cancer one is forced to conclude that the vast majority of cases of reported cure or improvement are to be regarded as more than questionable. The natural tendency of the disease to occasional remissions and to widespread and deceptive necroses, conjoined with the pardonable optimism of the therapistist, have given rise to a large amount of misinterpretation and of unfounded claims. After every allowance, however, has been made, there remains a very limited number of cases in which it seems permissible to conclude that a cure has actually been accomplished. In this category fall Stammler's case, Roving's cases, Bertrand's case, and possibly one case of Coca's. In addition to these cases of cure there are the numerous instances in which improvement in local and general conditions, and the prevention or delay of recurrence, have been described. It seems fair to admit that in many instances biological methods may be credited with at least a palliative value. Of the various methods employed it may be said that only one, namely, vaccination by means either of preserved unaltered tumor tissue, or of such tissues slightly altered by autolysis, has shown itself worthy of further trial; certainly it alone has the credit of even a slight degree of success. As opposed to the positive side of the picture must be weighed the difficulty and unpleasantness of the treatment, together with the enormous preponderance of negative results. As Ewing (53) states, "The complexity of the problem seems to bar the hope that a specific treatment of cancer will shortly become of practical importance." On the other hand, it is also true that the road has now been opened up to further investigation. It is certainly not oversanguine to express the hope that a prolonged period of patient work and cautious observation may eventually disclose a wider field of usefulness for the biological methods in cancer therapy. This consummation, however, can
OTHER BIOLOGICAL METHODS

only be delayed by the extravagant claims urged by certain partisan investigators in the past.

Finally it is important to emphasize the fact that animal experimentation should at every step guide the clinic. The spontaneous tumors of the lower animals differ in no important particular from those of man. The inoculable tumors provide an inexhaustible field for experimentation; here, it is true, the alteration in the biological relationship between the tumor and its host demands care and discretion in the interpretation of results. Nevertheless, with the exercise of the requisite caution and reserve, the results obtained in inoculable tumors have proven their availability in the study of the spontaneous disease. It is reasonable to believe that a method of treatment which would prove generally effective in experimental mouse cancer would also give the clue to an attack upon the human disease.

OTHER BIOLOGICAL METHODS

In addition to the methods above described, a variety of procedures, based upon more or less similar principles, have been advocated. It is not possible to describe all of these; indeed, the majority of them are already consigned to the limbo of obscurity. Of recent years more or less prominence has been achieved by Doyen's vaccines, by cancrin, and by antimeristem. In a special class is Coley's toxin.

Antimeristem is the name given by O. Schmidt to a pure culture of the supposed microbial agent of malignant tumors, belonging to the class of mycetozoa, to which are added the spores of the intermediate host, mucor racemosus. In an earlier form this preparation was described as cancrinoid. The asserted relationship of these organisms to the etiology of the disease has never been demonstrated. The therapeutic value of antimeristem when injected into cancer cases has been investigated by a number of authors—most carefully by Stockmann (121). It appears to be quite valueless.

Cancrin is the name given by Adamkiewicz (1) to a 25 per cent. watery solution of neurin, which is supposed to be identical with the toxic base of cancer. The discoverer ascribes to it healing virtues in cancer, which a multitude of other observers have never been able to duplicate.

Doyen described a small coccius, which he called microcococcus neoformans, as the cause of cancer. He prepared a vaccine with this organism, which was used in inoperable cancer, with the remarkable record of 20 per cent. of cures. Under Wright's direction the method was carefully tested out in the Middlesex Hospital, and found to be valueless.

The use of the toxins of the streptococcus was suggested to Coley by the observation that a case of sarcoma of the neck had been cured by an intercurrent attack of erysipelas. Similar observations have been reported by a number of others. Coley subsequently added the toxin of B. prodigiosus, both because it is in itself very powerful, and because the growth of the streptococcus appears to be stimulated by the presence of the prodigious bacillus. At the present time the mixed toxins are prepared, under Coley's direction, by Dr. Tracy, and are also supplied by Parke, Davis & Co. The method has been in use for over twenty years, and in 1913 Coley (40) reported that he had "upward of 70 successful cases, representing a little over 10 per cent. of successes of the total number treated."
cases comprise every variety, from the mildest, non-malignant types to the periosteal
sarcomas with practically fatal prognosis, in spite of wide removal.

Criticism of these claims has not been lacking. Bloodgood (23) has drawn
special attention to the difficulties in the microscopic diagnosis of certain cases
as between sarcoma and non-malignant connective tissue tumors. Moreover, there
is the curious tendency observed in some cases of so-called sarcoma to undergo
spontaneous cure, or regression, as observed by Klemperer, and admitted by
Coley himself.

In spite of these elements of doubt, it must be admitted that Coley can point
to a greater total of cures than can be claimed for any other similar method of
treatment. On the other hand, the X-ray has proven in competent hands to be
capable of causing the dissolution of a variety of sarcomata, either of the bones
(Pfahler), or of the soft parts. In the General Memorial Hospital we have seen
a large number of these tumors melt away under a few applications of the rays.
Radium also possesses the same power, though in less degree. The milder char-
acter of this treatment, together with its pronounced success, certainly recom-
mands it over the toxins, in the great majority of cases. It seems justifiable, how-
ever, to employ the toxins in all incurable cases in which the use of the X-rays is
impossible or ineffectual, and in cases of incomplete operative removal, under the
same conditions.

The most serious objection which can be urged against the treatment is its
severity. Against 10 per cent. of cures are to be balanced the infliction of repeated
chills, marked constitutional disturbance, and often severe pain, without benefit in
90 per cent. of the cases. Wright (139) has pointed out that the great defect of
the method is the absence of all information concerning "what changes are pro-
duced in the blood by successful inoculation, in order that these might be employed
in controlling and guiding the treatment." It is this lacuna, as Wright says, which
threatens completely to engulf all that is of value in Coley's method.

Among other observers who have obtained favorable results by its use may be
mentioned Friedrich (61), Petersen (103), Sproxton (119), Johnson (70), and
Répin (109).

Emmerich (52) and Scholl (116) substituted for the toxin the use of serum
derived from sheep which had been immunized by large doses of erysipelas cultures.
Their method does not appear to be good.

REFERENCES

    lxxix, 179.
REFERENCES

25. ——. Berl. klin. Woch., 1913, i, 1333.
33. Clowes. Immunity in Cancer, Publ. from Gratwick Lab., Buffalo, N. Y.
44. ——. Ibid., 1910, vii, 454.
47. ——. Zeitsch. f. Immun., 1910, iv, 531.
THE BIOLOGICAL TREATMENT OF CANCER

56. ———. Travaux de la deuxième conf., etc., Paris, 1910, 778.
57. ———. Lancet, 1911, ii, 1194.
58. ———. Tumori, 1913, iii, 16.
59. ———. Ibid., 1913, iii, 124.
61. Friedrich. Langenbeck’s Arch., 1895, i, 709.
1912, xxv, 278.
66. Héricourt and Richet. Deuxième cong. fr. de méd. int., 1895, ii,
600.
74. ——— and Meidner. Deutsch. Klin. am Eingang. d., XX Jahr-
hund., 1912, 41.
77. ——— and Hanes. J. Exp. Med., 1911, xiii, 505.
78. ——— ———. Ibid., 1911, xiv, 453.
83. ———. Ibid., 1911-12, xi, 317.
84. ———. Ibid., 1912, xi, 335.
REFERENCES

94. Metchnikoff. Ibid., 1900, xiv, 369.
101. ———. Studies from the Bender Hyg. Lab., 1906, iii, 5.
115. Salviati and De Gaetano. Rif. med., 1895, iii, 495, 507.
       1911, 2, p. 290.
139. Wright. In Discussion of Coley, No. 39.
CHAPTER XXXVI

THE TREATMENT OF GRAVES' DISEASE BASED ON SPECIFIC BIOLOGIC METHODS

M. MILTON PORTIS

As yet no satisfactory etiology has been advanced which will cover all cases of Graves' disease. The earliest theory attributed the cause to disease of the nervous system. Section of the restiform bodies will produce tachycardia, exophthalmus and hyperemia of the thyroid gland. Autopsies on cases of Graves' disease have shown changes in the medulla. The French, especially Charcot, attributed all the symptoms to disease of the sympathetic nervous system.

Moebius was the first to emphasize the thyroid gland itself as the exciting factor, and explained the disease on a basis of hyperthyroidism. There are many things in favor of the view of Moebius which is now generally accepted. The clinical picture in Graves' disease is exactly the opposite of myxedema, which all admit is due to lack of thyroid secretion. Again, feeding of the thyroid gland produces in a normal individual a picture simulating Graves' disease. If a case of Graves' disease is given thyroid gland or its derivatives, all the symptoms are intensified. Finally operative interference, either by ligating the blood supply or a partial thyroidectomy, in a case of Graves' disease at once lessens the symptoms.

Why does the gland become overactive? Many theories have been advanced. There seems to be a relationship between the thyroid gland and the sexual apparatus, for at puberty and during pregnancy a definite enlargement of the thyroid has been observed. This increase in size is associated with a hyperactive condition, which may go on and lead to the development of Graves' disease. An intoxication of intestinal origin is said to cause this thyroid pathology in some cases. Acute infections may precede and predispose to Graves' disease. Typhoid, influenza, syphilis, acute articular rheumatism, tonsillitis have all been forerunners of Graves' disease, and in certain cases at least, infection has been the exciting factor.

Grumme, on the other hand, believes, with Marimon, that Graves' disease and myxedema are due to hypothyroidism. Diseases of the thyroid are all due to various grades of hypothyroidism; a hyperthyroidism never occurs.

The occasional endemic appearance of Graves' disease has led some to suspect an infected water supply.
It may be that the cause which leads to hyperthyroidism is central and this in turn produces all the symptoms.

Cridle has forcibly shown that the phenomena of Graves' disease and those of the emotions, especially fear and anger, present striking resemblances. He emphasizes a reciprocal relation between the brain and the thyroid gland. The thyroid gland participating in emotional states may undergo a hypertrophy and this may lead to hypersecretion. In Graves' disease there is established a pathological stimulation of the entire motor mechanism. The nervous system, through the emotions, is capable of initiating such a motor excitation, but its continuance is effected through the secretions of the overactivated thyroid gland. We do not know why only certain individuals acquire Graves' disease when subjected to emotional strain. Cridle believes that "some reduced factor of safety, such as a disproportion of inorganic salts, or some general metabolic change accounts for the thin ice through which the exciting causes break." In support of his view is the repeated clinical observation that a great disappointment or an intense worry or fright may lead to the establishment of Graves' disease.

Marine doubts the primary thyroid hypothesis. He says: "We are in possession of more facts concerning the syndrome, as at present recognized, than can be harmonized with a primary thyroid hypothesis." He claims that the pathological changes are body-wide, although most marked in the thyroid. The thymus, the lymph glands, the spleen, the tonsils and the intestinal lymphoid tissues all show hyperplasia.

Wilson states that the pathological picture of the thyroid in Graves' disease varies with the age of the individual and the length of time the disease has lasted. He says that there is a close relationship between the clinical symptoms and the pathological condition of the gland. In general the picture is similar to that described by Halsted in experimental partial thyroidectomy. The part of the gland left undergoes a compensatory hypertrophy, especially of the secreting epithelium, which forms into papillomatous growths and fills the alveoli with cells. This is associated with an engorgement of all the vessels and a decrease in the colloid material.

In the earliest cases, according to Wilson, the functioning cells of the parenchyma swell and become columnar and the acini dilate with the secretion. Progressive hyperplasia follows, with overgrowth and stretching of the stroma. The acini are filled with infolding papillomatous growths of the epithelium, and contain a small amount of thin and slightly stainable secretion. An engorgement of all vessels is seen.

If the gland is from a case of eight months' to one year's duration, a similar picture is found, but the hyperplasia is less active, and more

1. Halsted has recently reviewed his earlier work. He now believes that infection occurred, which may have been the cause of the thyroid changes described.
secretion, which also stains more deeply, is found in the acini. This corresponds with a reduction of the intensity of the toxic symptoms. In cases of more than one year's duration the picture varies according to the clinical condition. We may find in parts of the glands either or both of the pictures described and, in other areas, atrophy and desquamation of the parenchymatous cells. The acini of such areas contain a deeply staining colloid material. If Graves' disease supervenes in a patient with an existing goiter, then its microscopic picture is added to the previous findings. However, islands of thyroid tissue will be found showing the picture described.

The lymph glands, especially those in the region of the thyroid, are often swollen and show hyperplasia. The entire lymphatic system, including particularly the tonsils, the papillae of the tongue, the spleen and the solitary follicles of the intestine, is involved in most cases. Most important and interesting is the persistence of the thymus in some instances. Hart believes that the thymus is frequently associated with Graves' disease and that it is responsible for some of the symptoms. He says that the hyperplastic thymus has toxic properties, which are neutralized by the thyroid. This overactivity in response to the demand, leads to hypertrophy and Graves' disease. Gebele, on the other hand, sees in the thymic hypertrophy a compensatory effort to offset the hyperthyroidism. This, however, does not account for cases without thymic enlargement.

The blood picture in Graves' disease shows a slight or moderate anemia, with frequently, as pointed out by Kocher, a leukopenia with a relative lymphocytosis. Capelle and Beyer have reproduced in animals the relative lymphocytosis by injection of emulsion of thymus. It is now generally accepted that the lymphocytosis is of thymic origin. The coagulability is often decreased, a fact which accounts for the frequent difficulty experienced in controlling hemorrhage.

Iodin is a constant and necessary constituent of the normal thyroid gland. It is found in many organs, but occurs in the thyroid in eight to ten times the amount found elsewhere. It plays an important rôle in the physiology and growth of the thyroid. The percentage varies directly with the amount of the colloid and inversely with the degree of hyperplasia, and the activity of the thyroid substance runs parallel with its iodin content. The iodin seems to be completely bound in a specific organic union, probably iodothyroglobulin. In Graves' disease, iodin occurs in lessened amount and in some cases only traces are found.

GENERAL TREATMENT

A great diversity of opinion still exists in regard to the treatment of Graves' disease. This is not surprising when we consider that as yet no satisfactory etiology has been advanced for this bizarre symptom-complex.
The most important of non-operative measures is rest. To be effective one should individualize and not forget that the rest must be mental as well as physical. The nervous and circulatory excitement yield readily to this measure. In a severe case, isolation in a quiet, airy room, with a Weir Mitchell rest cure, is of decided benefit. An ice-bag or Leiter coil should be applied over the heart and, in a stubborn case, over the thyroid as well. In mild cases, a vacation in the country or at the seashore will prove useful. The period of rest must be at least six weeks or longer. It requires the same length of time to recover from Graves' disease as from a nervous breakdown of other origin.

Some type of toxin originates in the alimentary tract in the digestion of meat which must be neutralized by the thyroid. In carnivorous animals we find relatively larger thyroids. This detoxicatory property of the thyroid was clearly shown by Blum. He demonstrated that thyroidectomized dogs, which were doing well on milk, developed symptoms of athyroidia as soon as they were fed on meat. Jacquet and Svenson have shown that, in dogs fed on meat in large amount, the thyroid becomes very poor in iodin, probably owing to the rapid consumption of the thyroid secretion to accomplish the neutralizing demands. Meat, therefore, is to be avoided in all cases if one wishes to avoid stimulation of the already over-functionating gland in Graves' disease. Milk alone, or milk diluted with lime water, or, better still, fermented as Bulgarian buttermilk, should be the chief article of diet. Besides milk, vegetables thoroughly well cooked, and fruit, especially stewed, are permitted. Later, when the disease is yielding to treatment, other articles may be gradually added. Alcohol, tobacco, coffee and tea are excluded.

A host of drugs have been recommended, the majority of which have no value. The natural tendency of the disease to undergo spontaneous remissions has given to some of the drugs a reputation which they do not merit. Small doses of the bromids, especially the neutral quinin hydrobromid, is often of considerable service in controlling the nervous excitability and the tachycardia. Iron, combined with a mild laxative, is indicated in the anemic cases. The tincture of strophanthus seems to exert a better influence on the tachycardia, than does any other drug of the digitalis group. The myocardial degeneration of the chronic cases is to be treated on the usual lines for cardiac diseases. Thyroid extract in small doses may be tried cautiously in those cases where one suspects that the overactivity is at first a defensive reaction, but in most cases it will aggravate the symptoms, and, in general, this practice is extremely unsafe. Extracts of other ductless glands, including the thymus, adrenal and hypophysis and also of the ovary and testicle have all been tried with no benefit.

The local application of remedies such as iodin, mercurials, Faradism, galvanism, aside from their psychic effect, do not accomplish very much.
TREATMENT WITH SERA

Massage of the body and hydrotherapy are useful for their general tonic effect. The X-ray over the thyroid and over all cases with a persistent thymus has shown in some cases real benefit. It should be tried in all stubborn cases. The lymphocytosis disappears quickly after a few such treatments over the thymus. Injections into the gland of alcohol and carbolic acid to destroy part of the thyroid and thereby lessen its secretion have been recommended. Porter uses for this purpose hot water. This treatment has not become popular.

TREATMENT WITH SERA

In the serum therapy of Graves’ disease two types are to be considered: an antitoxic and a thyrotoxic serum. Based on the idea of detoxicatory function of the normal thyroid and, that, in the absence of the thyroid, such substances must accumulate which normally combine with the thyroid secretion to make it innocuous, Moebius used a serum of thyroidectomized sheep. This serum given in ascending doses has seemed efficient in some cases. A serum of thyroidectomized goats based on the principle of Moebius, under the trade name of thyroidectin, was used extensively, but with uncertain results. The use of milk of thyroidectomized goats is very popular in Europe. A dried product of such milk is on the market under the name of rodagen. The fresh milk taken in large amount has some value, especially as a food, but the small amount represented by the usual dose of the dried powder can have but little influence. A serum of thyroidectomized horses, in increasing doses by mouth, has given decided benefit at times. Certain cases treated in this manner by the writer have seemed to react favorably.

The brilliant results demonstrated by a partial thyroidectomy led the writer, ten years ago, to attempt experimentally to produce a serum that would destroy the thyroid in situ. Experiments were conducted in which the thyroid glands of dogs were injected intraperitoneally in goats. In the later experiments, thyroids freed from blood were used and, still later, colloid matter alone. The following is an extract from the summary of results reported:

The serum of goats injected with suspensions of the thyroid gland or with thyroid colloid material of dogs acquires many new and striking properties. Injected into dogs, it causes marked symptoms, prominent among which are depression, convulsions, vomiting, and rapid breathing, hemoglobinuria, and early death in some cases, and in other animals that lived longer there was present also some fever, lachrymation, loss in weight and progressive weakness. It cannot be claimed that there has been reproduced the exact picture presented by thyroidectomized dogs.

These clinical manifestations are associated with removal of colloid matter from the acini of the thyroid gland, desquamation and disintegration of the epithelial cells of the acini, followed in time by processes of repair and growth.
of papillary proliferations. The parathyroid bodies and hypophysis show no changes. The liver, spleen and kidneys present market degenerative changes.

The toxic serum is markedly agglutinating and hemolytic for dog's corpuscles even when obtained from colloid matter. If it were possible to remove from the thyrotoxic serum the hemolytic as well as other direct and indirect cytotoxic actions, it would seem warranted to expect still further evidence of a specific thyrotoxin.

To overcome the difficulties in the use of an emulsion of the entire thyroid cell in the production of an antiserum, Beebe and Rogers were the first to suggest the use of chemical substances derived from the gland. They based their experiments on the idea that nucleoproteins derived from nuclei are the most specific of all the cell constituents and that specific sera can be produced by their use as antigens. At first they used glands removed from cases of Graves' disease, but later used normal human thyroids from recent autopsies. The nucleoproteins thus prepared were injected into Belgian hares and rams and a potent serum obtained. They have reported a large series of cases treated successfully by small hypodermic doses of their serum.

Pearce and Jackson repeated their experiments and did not obtain a serum which they regarded as specific. Taylor used a serum prepared according to their method in a number of cases without any benefit. Wells was unable to demonstrate any antigenic action with nucleoproteins, and a careful chemical study of such products by him throws considerable doubt on the possibility of obtaining any specific nucleoproteins at all by the present methods. Wells observed that with repeated precipitations performed for the purification of nucleoprotein, the antigenic property of the product became less and less. The writer, with Dr. Bach, has just published the results of a series of experiments which confirm the work of Pearce and Jackson and Wells. A serum carefully prepared in every detail according to the method of Beebe did not show any specificity for thyroid, but was rather general in its action on other organs, especially the liver, spleen and kidney. The antibodies produced were hence ascribed to the small amount of protein contained in the injected material.

When the last word has been said, in the light of our present knowledge, we still must admit that we are not able to cure all cases medically. Surgery has demonstrated more brilliant results than has medicine so far. But not all cases are surgical. The mild cases, the cases with acute symptoms, the advanced cases with cardiac degeneration, the cases that although operated on have not improved, all these should be treated medically.

The internist should not temporize with cases that resist medical measures or cases with relapse of acute symptoms. These are surgical, and they should be operated on when the acute symptoms are under control and before irreparable degenerative processes have occurred.
CHAPTER XXXVII

NORMAL SERA AND BLOOD IN THE TREATMENT OF ANEMIA AND
THE HEMORRHAGIC DISEASES

G. H. WHIPPLE AND W. L. MOSS

THE MECHANISM OF COAGULATION OF THE BLOOD

G. H. WHIPPLE

The group of hemorrhagic diseases is a very unsatisfactory and
indefinite one. We may include here almost any disease with which pur-
pura or bleeding is an important symptom. The tendency to hemorrhage
is a symptom and not a true disease, and, like icterus, it is a symptom of
a disease which affects some organ or tissue of the body. Some of the
clinical entities are well recognized, and must be designated by their
familiar names whether suitable, confusing, or otherwise.

The theories of blood coagulation are many and varied, and need not
be reviewed. It is quite essential, however, to have clearly in mind the
mechanism of normal blood coagulation. The theory of Howell meets the
known requirements of blood coagulation in health and disease in the most
satisfactory manner, and we may accept this as a working hypothesis until
it is shown inadequate.

\[
\begin{align*}
\text{Antithrombin} & \quad \text{→} \quad (\text{Thromboplastin}) \\
\text{Prothrombin} & \quad \text{→} \quad (\text{Thrombin}) \\
\text{Calcium} & \quad \text{→} \quad (\text{Fibrinogen}) \\
\text{Fibrinogen} & \quad \text{→} \quad (\text{Clot})
\end{align*}
\]

The substances included in parentheses are not present in the circulat-
ing blood. The prothrombin is held in an inactive state by the anti-
thrombin which can be demonstrated in normal blood. Thromboplastin
is freed by any tissue injury (blood cells, plates, endothelium, etc.), and
neutralizes the antithrombin, thus freeing the prothrombin. Coagulation
then occurs by formation of thrombin and precipitation of the fibrinogen.
The logical method of classification and study of various types of hemorrhagic disease is to group them under the headings indicated in the schema given above for blood coagulation. This method has disadvantages, but also some advantages, as one is forced to look at a disease from a different viewpoint, which in itself may be helpful.

**Fibrinogen.**—This element fluctuates widely in amount in man and animals, but in health never falls to a dangerously low level. (Whipple, 22.) Its rate of regeneration in health is extremely rapid (Goodpasture, 5), and the reserve capacity of reproduction by the body seems limitless. This in itself indicates the great importance of the protein in the body economy. It may be greatly depleted by various poisons (chloroform, phosphorus) which injure the liver, and in severe poisoning the fibrinogen may practically disappear. This explains the disseminated ecchymoses, gastric hemorrhage, and bleeding noticed in such cases. The clots are too flabby to close any ruptured vessels. The hemorrhagic symptoms of acute yellow atrophy and yellow fever are referable to this drop in the blood fibrinogen to a very low level due to liver injury. Various chronic liver diseases (cirrhosis) may show a low fibrinogen index, and this is of very serious prognostic importance. This low fibrinogen index will favor hemorrhage. It is to be kept in mind, however, that liver disease may be associated with normal fibrinogen, but with abnormalities in other factors of coagulation.

From a theoretical standpoint there is no reason to expect any favorable reaction from serum treatment in such conditions. Whole blood might help to tide a patient over a period of acute fibrinogen insufficiency until regeneration of the liver cells can adjust the normal balance.

**Calcium.**—There is no evidence that any form of hemorrhage is referable to abnormality in this element. Icterus may show delayed coagulation time, which may be improved by calcium feeding, but in such cases the calcium blood content is above normal. It is probable that the calcium is bound by the bile pigments, and is only slowly available for the requirements of coagulation. There is no serious danger in this condition. True hemorrhagic symptoms with icterus may be associated with other abnormalities in blood coagulation (Whipple, 23), and are considered below.

**Prothrombin.**—This elusive element is rarely involved in hemorrhagic disease. Hemorrhagic disease of the newborn in some, perhaps all, cases is associated with disappearance of this substance from the circulating blood. (Whipple, 23 and 24.) There is good evidence that the prothrombin may be present at birth, but vanishes during the first few days of life. It is obvious that fresh serum which is rich in thrombin should be of value, and experience has confirmed this. Pure thrombin should be the ideal treatment. Hemophilia, according to recent work of Howell (9), shows a lowering of the prothrombin content of the blood plasma. Theoretically, then, one would expect help from serum injections.
Antithrombin.—The antithrombin-prothrombin balance is in very delicate equilibrium, and can be upset by various experimental procedures—for example, intravenous injection of peptone—but the capacity of the normal body to readjust this disturbed equilibrium is very great. It is pretty clear that the liver may be concerned with the production of antithrombin, and perhaps its destruction, but it is also certain that thrombin can in some way be neutralized in the blood outside of the liver. It is not surprising, then, that in disease one may meet with hemorrhagic symptoms, or periods which are due to excess of the antithrombin factor. This has been shown (Whipple, 23 and 24) to be true in certain cases of septicaemia, miliary tuberculosis, endocarditis, etc. It is possible that the rapid tissue destruction and disintegration may have freed substances capable of stimulating the liver to an overproduction of antithrombin. Another group of cases, leukemias and anemias, may show the same abnormality. This may be found in aplastic anemia with complete marrow aplasia, showing that the reaction of the bone marrow is not a factor in this complex.

Diseases of the liver with icterus may at times be associated with an antithrombin excess and develop grave hemorrhagic symptoms. It is obvious that calcium would be of no therapeutic value in cases of icterus with bleeding of this type. Cases of this type with mild icterus may develop for no apparent reason, and after a period in which bleeding may be troublesome and dangerous may suddenly return to normal without treatment of any kind. This fluctuation in the antithrombin content is quite obscure.

Other Factors.—It has been suggested that some types of hemorrhagic disease may be referable to increased fragility of the capillaries. This is simply an evasion of the point at issue, and no direct evidence has ever been adduced to support this view. Fewer and fewer cases will be grouped here as more definite data are accumulated to show the real cause of the bleeding.

Blood platelets are known to fluctuate in disease, and it has been suggested by Duke (3) that a great drop in the number of blood plates may favor bleeding and purpura. It is possible that other elements of blood coagulation may fluctuate in a like fashion. Howell (9) has reported cases of purpura in which no abnormality of blood coagulation was demonstrable, but the blood plates were not counted.

Fibrin-dissolving ferments may be concerned in some cases of hemorrhage, even in fatal cases in adults. This ferment may be very active, and can dissolve blood clots in vivo or in vitro with great rapidity. Consequently, even with normal elements of blood coagulation, the clots are not permanent, and oozing continues through the softened clots which form at the site of injury. This ferment may be present in small amounts (Goodpasture, 6) in cases with liver disease, even if not sufficient to give rise
to hemorrhagic symptoms. Normal blood plasma contains a ferment capable of inactivating this fibrinolytic ferment.

CLASSIFICATION AND TREATMENT OF THE ANEMIAS AND HEMORRHAGIC DISEASES

W. L. Moss

The blood is a fluid so essential to life that it is not strange that physicians in every age have sought to influence disease through this medium. The history of therapeutics from its earliest days abounds in the records of these attempts at blood therapy. The blood has been depleted by bleeding, cupping, leeching, purging, sweating; and efforts have been made to augment or otherwise alter it by the introduction of normal and abnormal blood from man and beast. Some of these methods are founded on a rational basis, and their proven value entitles them to a place in our present-day therapeutics; others are only of historical interest.

In recent years there has been such a revival of interest in the efforts to treat disease by means of the introduction of blood or its various constituents, and in some instances at least with such a measure of success that no treatise on therapeutics is complete without a discussion of the subject.

The use of the various immune or specific sera has been considered elsewhere in this volume, and the present chapter deals with the use of normal blood and its derivatives in the treatment of disease. The diseases to which this form of therapy has been applied consist mainly of the anemias and a large group of diseases in which hemorrhage may occur. From the latter group there may be separated a smaller group somewhat loosely designated as the hemorrhagic diseases.

A satisfactory classification of the anemias cannot be made, owing to our incomplete knowledge of their etiology. They are usually divided into primary, or essential, and secondary. "By primary is meant one for which an adequate cause cannot be assigned. By secondary anemia is meant one for which the cause assigned seems adequate to explain the blood condition." (Emerson, 4.)

Under primary anemias Osler (19) mentions only two diseases: chlorosis and idiopathic or pernicious anemia. Many authors include here also the leukemias, Hodgkin's disease, and splenic anemia.

Some confusion has arisen from the use of the terms primary type of anemia and secondary type of anemia. By the former is meant an anemia with a high color index, the latter is used to designate an anemia with a low color index. Thus chlorosis, which, on the basis of etiology, is classed as a primary anemia, is, on the ground of the color index, one of the best
examples of the secondary type of anemia, and not infrequently carcinoma
of the stomach leads to an anemia with a color index above one.

We have attempted to make etiology the basis of the following classifi-
cation; it is, of course, tentative, and the primary group will diminish as
the causes of the diseases included in it are discovered. Until recently we
would have included Hodgkin's disease with the primary anemias, but
such strong evidence of the bacterial nature of the disease has been fur-
nished by Negri and Mieremet (18), and Bunting and Yates (1), that we
have placed it with the secondary anemias. The classification of the sec-
ondary anemias is taken from Osler (19).

**Anemia**

**Primary or Essential Anemia**

1. Chlorosis.
2. Idiopathic or Pernicious Anemia. Sub-type—Aplastic An-
   emia.
3. Leukemia.
   (a) Myeloid or Splenomedullary.
   (b) Lymphoid or Lymphatic.
4. Splenic Anemia (Banti's Disease).

**Secondary Anemia**

1. Acute Secondary Anemia. Hemorrhage, certain acute infections,
   and intoxications are the important causes.
2. Chronic Secondary Anemia; of which the important causes are:
   (a) Inanition: due to defective food supply, unhygienic surround-
       ings, chronic dyspepsia, cancer of esophagus and stomach.
   (b) Infections: especially typhoid fever, rheumatic fever, sepsis,
       syphilis, malaria, ankylostoma, and bothriocephalus.
   (c) Intoxications: inorganic poisons, such as lead, mercury, arsenic;
       organic poisons, such as the toxins of various fevers; and certain autog-
       enous poisons occurring in chronic affections such as nephritis, jaundice.
   (d) Hemorrhage: repeated hemorrhages, even though small, such as
       the persistent bleeding from hemorrhoids.
   (e) Long-continued drains upon the system as in chronic suppura-
       tion, prolonged lactation, and rapidly growing tumors.

The difficulty in classifying the diseases with which hemorrhage may
be associated is as formidable as that met with in the case of the anemias.

Under the designation Hemorrhagic Diseases we have separated a
group whose striking and important characteristic is a tendency to im-
moderate hemorrhage. The limits of this group are at present not very
clearly drawn, but it should probably include only those diseases in which
the tendency to bleed is dependent upon some disturbance of the factors concerned in the coagulation of the blood. If this is made the basis of the classification it will appear from the preceding discussion of the theories of coagulation that the group may be divided into sub-groups, depending upon the particular factor or factors which may be at fault. Thus in one group might be included those diseases in which the hemorrhagic tendency depends upon a deficiency of prothrombin, another might include those in which there was an excess of antithrombin, a third might include those diseases in which the hemorrhage is due to deficient fibrinogen, and so on for each of the factors concerned in coagulation. Of course it is highly probable that the conditions are too complex to fit into any such simple classification as suggested above. It is probable that two or more factors may be disturbed simultaneously in some instances, and even possible that in a given disease the same factors are not always at fault.

We have separated from the hemorrhagic diseases a large heterogeneous group which we have designated Diseases with Which Hemorrhage May Be Associated. This group includes a number of infectious diseases due to bacteria, those due to animal parasites and those of unknown etiology. It also includes a variety of non-infectious diseases.

In some of the diseases of this group the anatomical lesions present seem adequate to explain the hemorrhage, and in such cases it is not necessary to presuppose the existence of any disturbance of the factors influencing coagulation. Thus in some instances the erosion due to ulcers in the stomach or intestines, the ulceration of neoplasms of the alimentary tract, genito-urinary system, and elsewhere, renal tuberculosis, or the presence of stone in the kidney or bladder may readily account for hemorrhage. But even in these easily explicable cases it seems likely that if the hemorrhage is sufficient to cause a marked grade of anemia which persists for a considerable length of time, there may be secondary changes in the blood leading to a disturbance in its coagulability which may prolong the hemorrhage. It seems not improbable, even in typhoid fever, a disease in which the intestinal hemorrhages are usually ascribed to the erosion of vessels by ulcers, that in many cases the important underlying cause of the hemorrhage is a disturbance of the balance between the factors upon which coagulation depends. The same may be true of the hemorrhage in certain cases of tuberculosis.

In other diseases included in this group, septicemia, diphtheria, variola, scarlet fever, measles, typhus fever, yellow fever, scurvy, and acute yellow atrophy, the hemorrhagic tendency is not so easily explained, and is rather vaguely considered to be toxic in origin.

The desirability of a knowledge of etiology for the classification of the hemorrhagic diseases has already been pointed out; it is even more desirable for the treatment.

There have been no studies, so far as we know, in which all of the
factors influencing coagulation have been investigated simultaneously. A number of observers have followed one or several of the factors, and such data as are available indicate that a disturbance in certain factors may be characteristic for a given disease, but the observations have been so incomplete, and the series of cases so small, that generalizations would be unsafe.

Rather than attempt a classification on an etiological basis, which would not only be incomplete, but almost certainly faulty, it seems wiser to refer very briefly to the findings in the few cases which have been at all carefully investigated, and trust that the recognition of the sort of studies that are necessary to advance our knowledge on this important subject will stimulate investigators to further work in this field.

Hemorrhagic Diseases

1. Hemophilia.
2. Morbus Maculosus Neonatorum.
3. Purpura.
   (a) Purpura Simplex.
   (b) Purpura Rheumatica.
   (c) Purpura Hæmorrhagica.
4. Essential Hematuria.

Diseases with Which Hemorrhage May Be Associated

Typhoid Fever.
Septicemia.
Diphtheria.
Pertussis.
Dysentery, Bacillary and Amebic.
Plague.
Tuberculosis.
Malaria.
Relapsing Fever.
Syphilis.
Pulmonary Distomiasis.
Bilharziosis.
Filariasis.
Variola.
Varicella.
Scarlet Fever.
Measles.
Typhus Fever.
Yellow Fever.
Dengue.
SERA IN THE TREATMENT OF ANEMIA

Rocky Mountain Spotted Fever.
Plumbism.
Pellagra.
Scurvy.
Cirrhosis Venticuli.
Gastric and Duodenal Ulcer.
Ulcerative Enteritis and Colitis.
Cancer of Alimentary Tract and Genitourinary System.
Diseases Associated with Jaundice.
Hepatic Cirrhosis.
Nephritis.
Pernicious Anemia.
Leukemia.
Splenic Anemia.
Epistaxis Due to Local Causes.
Genitourinary Conditions Due to Stone, Neoplasms, and Infections.
Diseases of the Female Generative Tract.

METHODS OF TREATMENT

Since we are going to consider relatively few agents which may be applied in the treatment of a great variety of conditions, it will save much repetition to describe the agents employed, their source, preparation, properties, mode of action as far as known, and methods of administration, before discussing their prophylactic and therapeutic application. The agents are: (1) normal serum (in contradistinction to immune), (2) defibrinated blood, and (3) whole blood. Either human or animal serum may be employed, but when defibrinated blood or whole blood is used it should be of human origin.

In the use of human blood or serum care should be exercised that the donor is a strong, healthy individual, or at least one free from communicable disease. Syphilis especially should be excluded, not only by a negative history, but, when practicable, by a negative Wassermann reaction.

Serum

A variety of sera have been employed; for instance, horse, sheep, goat, beef, rabbit. Beef and goat sera are said to be more toxic than the others, and on that account their use is less desirable. Although normal horse serum may be obtained from a number of the large drug houses which manufacture antitoxins, it would be difficult to get it as promptly as might be necessary, or as fresh as it seems desirable to use it. Another objection to its use is the possible danger from anaphylaxis in a patient who has previously received antitoxin (horse serum), or of sensitization in one who
might subsequently develop the need for antitoxin. Although the danger from anaphylaxis has probably been greatly exaggerated, it seems wiser to avoid the risk when possible. Good results in the treatment of hemorrhage have been reported from the use of antitoxic serum, but it is doubtful if this agent is as useful as fresh serum.

The rabbit furnishes the most convenient source of fresh supply, and its serum is not only without toxicity in the doses employed, but appears to be the most efficacious of the animal sera in the treatment of hemorrhage.

To Obtain Rabbit Serum.—A large, healthy rabbit is selected, anesthetized, the front of the thorax is shaved and the skin rendered aseptic. Blood is aspirated from the heart through a needle of fairly large caliber by means of a sterile 20 c. c. syringe. The needle is inserted at a point about 1 cm. to the left of the midline and 1 cm. above the level of the costal angle, being directed upward and toward the midline. Usually as much as 60 c. c. of blood may be obtained from a good-sized rabbit without sacrificing the animal. If more than 20 c. c. of blood is desired it is convenient to use a needle which is attached to the syringe by means of a push connection rather than a screw connection. After the syringe has been filled it is detached from the needle, which remains in situ, and the blood is transferred to a sterile centrifuge tube. A second, and even a third, aspiration of blood may usually be made with the same syringe if one works rapidly, but it is well to have a second syringe ready in case the blood begins to coagulate in the first. As soon as the blood is coagulated the clot is detached from the sides of the centrifuge tubes by means of a sterile platinum needle, and the serum is allowed to separate. After one to two hours the tubes may be centrifuged and the serum removed by means of a sterile pipette. If the serum is intended for intravenous injection it should be entirely free from cells. These may be removed, if present, by further centrifugation. If it is to be injected subcutaneously the admixture of a few cells does no harm.

To Obtain Human Serum.—If only a small quantity is desired the blood may be aspirated from one of the large veins at the bend of the elbow by means of a syringe. In case a larger quantity is desired than can be obtained conveniently with a syringe one may employ an aspirating outfit made in the following way: A 100, 200, or even 250 c. c. glass cylinder is fitted with a rubber stopper, through which are passed two glass tubes about three inches long, bent at the middle to a right angle. To the outer end of one of these tubes is attached a short needle of fairly large caliber by means of a rubber tube one or two inches long. To the other glass tube is attached six or eight inches of thick-walled rubber tubing, ending in two inches of glass tubing, which serves as a mouthpiece. This apparatus is sterilized by boiling, and before use a little sterile cotton is pushed into the mouthpiece to prevent the access of bacteria. A bandage
is placed around the upper arm of the person from whom the blood is to be obtained, sufficiently tight to cause the veins to stand out prominently, but not tight enough to obliterate the radial pulse. The skin having been previously cleaned, the needle of the aspirating apparatus is inserted into a vein and the flow of blood into the cylinder accelerated by suction applied through the opposite tube. After the desired amount of blood has been obtained the bandage is removed from the arm, the needle withdrawn from the vein, and a sterile sponge quickly placed over the puncture wound, and moderately firm pressure applied for a half minute to a minute to prevent the possible formation of a hematoma.

The rubber stopper in the cylinder is replaced by a sterile cotton plug, and as soon as the blood has coagulated the clot is separated from the sides of the cylinder by means of a small sterile glass rod. The serum is allowed to separate, and after several hours is removed by means of a sterile pipette and rubber bulb.

**Properties of Serum.**—Normal serum differs from whole blood in that it contains no cellular elements, although it may contain substances (thromboplastin?) liberated by the disintegration of platelets and leukocytes. It contains no fibrinogen, no antithrombin, and less calcium salts than the blood. It contains no prothrombin, but free fibrin ferment (thrombin), which is not present in whole blood. Morawitz (14) and others have shown that on standing a few days thrombin is converted into an inactive form, metathrombin. This may explain the better results following the use of fresh serum.

**Action of Serum.**—Clinical results leave little doubt that serum, administered subcutaneously or intravenously, is a valuable hemostatic in some cases of hemorrhage. Also that it may be a valuable prophylactic agent before operation in individuals with a hemorrhagic tendency, but we are as yet ignorant of its mode of action. It has been used fairly extensively, and the accumulated experience indicates that there is little, if any, danger of producing intravascular clotting.

Howell (8) has shown that large amounts of serum, and even of pure thrombin, may be injected intravenously in animals without apparent injurious effects. The antithrombin content of the blood may show an increase a few hours after such injections, but quickly returns to normal. This increase in antithrombin might be regarded as a contraindication to the use of serum in cases where the hemorrhagic tendency depends upon an excess of antithrombin, and the same might apply to the use of defibrinated blood. It should be taken into consideration, however, that these observations were made upon animals whose blood was presumably normal as regards the factors influencing coagulation, and it is not certain that they would apply to human beings whose hemorrhagic tendencies lead us to presuppose some disturbance of these factors. While emphasizing the
value of such observations, and the importance of any study that will throw light on the mode of action of these agents, we feel that the question of their usefulness will be determined on a basis of clinical results.

We are unable to say whether animal serum or human serum is more efficacious in the treatment of hemorrhage.

**Methods of Administration.**—Serum may be given subcutaneously in doses of 10 to 30 c. c., or intravenously in doses of 10 to 15 c. c. It is apparently more prompt in its action and more efficacious if given intravenously. Sometimes a single dose suffices to stop the hemorrhage. In case of continued bleeding the dose may be repeated at intervals of two to six hours, or even longer, depending upon the urgency of the indications. If the bleeding is not controlled by the first few administrations of serum little good can be expected from its continued use. There is no danger from anaphylaxis attending the use of human serum. In case animal serum is used it is advisable to ascertain whether the patient has ever received a previous injection of serum from the animal species to be used. The danger from anaphylaxis attending intravenous injection is greater than that from its subcutaneous use. There is no danger from anaphylaxis when the last injection is made within seven days of the first injection. If necessity should arise for further serum treatment after a lapse of more than seven days from the first serum injection it would be wise to use serum from an animal of a different species.

**Defibrinated Blood**

Defibrinated blood may be given subcutaneously in small amounts, or intravenously in amounts up to 600 c. c. It differs from whole blood in that the platelets and, to some extent, the leukocytes have been destroyed, but, as in the case of serum, it may contain some of the disintegration products (thromboplastin?) of these cells. It has been deprived of its fibrinogen and antithrombin, and the amount of calcium salts has been reduced. The prothrombin has disappeared, and it contains free fibrin ferment.

**Mode of Action.**—Defibrinated blood in small amounts subcutaneously or intravenously would appear, a priori, to have the same action as serum similarly introduced, except for any additional action which may be due to the presence of the red blood cells. A discussion of this subject will be deferred until we come to consider the treatment of pernicious anemia. Large amounts of defibrinated blood have been employed intravenously in place of direct transfusion in a variety of conditions. Experimental results indicate that the red blood cells introduced are able to live and functionate in the patient's circulation. The presence of the large amount of thrombin is apparently well tolerated. The observation previously mentioned, namely, that the introduction of thrombin stimulates the body
to the production of an excess of antithrombin, might be considered a con-
traindication to the use of this method in patients where the hemorrhagic
tendency is dependent upon an excess of antithrombin, and the method
may prove useless in those cases where the faulty coagulation depends
upon an absence or deficiency of fibrinogen. Apart from these theoretical
objections, the value of the procedure will probably ultimately be deter-
mmed by the clinical results.

It should be noted that the introduction of defibrinated blood is fre-
quently followed by a febrile reaction on the part of the patient. This
usually begins within an hour, and may be accompanied by a chill. The
temperature may reach 103° F., or higher, but falls to normal in a few
hours. This reaction does not seem to detract in any way from the value
of the procedure.

Preparation of Defibrinated Blood.—To obtain small amounts blood
is aspirated from an arm vein of the donor by means of a syringe, and
transferred to a sterile flask containing glass beads, and shaken for ten
minutes. If it is for intravenous administration it should be filtered
through several layers of sterile gauze after defibrination. This precaution
may be omitted in case of subcutaneous injection.

Intravenous Administration of Large Amounts of Defibrinated Blood.
—One of us has described a simple technique for indirect transfusion, the
details of which may be found on reference to the original article (15).
Briefly, the procedure may be described as follows: The apparatus for
obtaining and defibrinating the blood consists of several Erlenmeyer
flasks of 300 c. c. capacity, each containing about one ounce of glass beads
and stoppered with cotton, a rubber stopper through which are passed two
short glass tubes, to one of which is attached a short needle of moderately
large caliber, to the other six or eight inches of thick-walled rubber tubing,
ending in a mouthpiece, as described above.

The flasks are sterilized by dry heat, the rest of the apparatus by
boiling. Previous to use the inside of the needle and attached tube of the
aspirating outfit is coated with sterile paraffin. The stopper carrying the
needle is then fitted to one of the flasks containing glass beads, and the
blood is aspirated from an elbow vein of the donor. When about 200 c. c.
of blood has been obtained the flask is removed from the stopper without
disturbing the needle in the vein, another flask is substituted in its place,
and more blood aspirated. The above procedure is repeated until the
necessary amount of blood is obtained. As soon as each flask is filled it is
stoppered with a plain rubber stopper and shaken for ten minutes to de-
fibrrinate the blood. For an adult the optimum amount of defibrinated
blood appears to be about 500 c. c. This amount is readily obtained from
600 c. c. of whole blood. The defibrinated blood is next filtered into an
infusion bottle through several layers of sterile gauze and allowed to flow
by gravity into a vein of the patient.
Whole Blood

Intravenous Use of Whole Blood.—Various methods of direct and indirect transfusion of whole blood are in use, the technique of which need not be described here. Direct transfusion has proved a life-saving measure in numerous instances, but with the development of adequate methods for indirect transfusion of both whole and defibrinated blood one may look to the discontinuance of a method which is available only when the services of a skilled surgeon who has had considerable experience in carrying out the procedure are at hand.

The more obvious disadvantages of direct transfusion are the difficulty of the technique, the necessary scar resulting in both donor and donee, the permanent obliteration of blood vessels, the impossibility of determining the amount of blood transfused, the difficulty of regulating, with any degree of accuracy, the rate of flow, and thus avoiding the consequent danger, even though slight, of acute dilatation of the heart.

With the recognized importance of transfusion we may confidently expect the development of a satisfactory technique for indirect transfusion of whole blood. Linderman (11) has recently published a method which consists in introducing a specially designed cannula into a vein of the donor and a similar cannula into a vein of the patient. By means of a large number of 20 c. c. syringes blood is withdrawn from the donor and injected into the patient, a fresh syringe being used for each transfer of blood.

Kimpton and Brown (10) proposed a method for indirect transfusion of whole blood. The apparatus consists of a glass cylinder of 200 or 300 c. c. capacity, the lower end of which is drawn out into a small tube bent at right angles to the axis of the cylinder. The inside of the cylinder and tube is coated with paraffin to prevent coagulation. The end of the tube is introduced into an arm vein of the donor and blood allowed to flow into the cylinder under the heightened venous pressure produced by a bandage around the upper arm. The tube is then removed from the donor’s vein and introduced into a vein of the patient. The introduction of the blood into the patient is brought about by pumping air into the upper end of the cylinder. Great care must be exercised that no air is introduced into the patient’s vein.

In the case of direct or indirect transfusion of either whole blood or defibrinated blood it is important, where possible, to select a donor who belongs to the same iso-agglutinin group as the donee. Methods for making this determination have been described elsewhere (16, 17). The test may be carried out in the absence of known groups as follows: A few drops of blood are collected from the ear or finger tip of the patient in a glass tube, as for the Widal reaction, and allowed to coagulate in order to furnish serum. An additional drop or two of blood is allowed to fall into
a centrifuge tube containing a few cubic centimeters of 1.5 per cent.
sodium citrate solution in 0.85 per cent. sodium chlorid solution. The
corpuscles thus obtained are washed twice in normal salt solution and
then brought to approximately a 1 per cent. suspension in normal salt
solution. In a similar way serum and corpuscles are obtained from the
prospective donors. The agglutinating action of the serum of the patient
is tested against the corpuscles of each of the prospective donors, and the
serum of each of the donors is tested for its agglutinating action against
the corpuscles of the patient. This test may be made in the hanging drop
by adding a small drop of the serum to an equal quantity of the suspension
of corpuscles. The presence or absence of agglutination may be observed
under the microscope. If the serum of individual A does not agglutinate
the corpuscles of individual B, and if B’s serum does not agglutinate A’s
corpuscles, the two individuals belong to the same iso-agglutinin group.
It is not necessary to test for isoemolysins, since it has been shown that
isoemolysins, when present, follow the same laws which govern iso-ag-
glutination.

APPLICATION OF THE METHODS OF TREATMENT

Primary Idiopathic Anemia

Chlorosis.—Rarely the degree of anemia in this condition may reach
an extreme grade, but the response to general hygienic measures and the
administration of iron are so satisfactory that the necessity of resorting to
any of the methods of treatment considered in this chapter would scarcely
arise.

Pernicious Anemia.—The frequency with which this condition resists
the usual therapeutic measures has ever led clinicians to try new measures
with the hope of obtaining more satisfactory results. The usual type of
pernicious anemia is characterized by remissions, during which there is
improvement, but followed sooner or later by relapse, and eventually a
fatal termination. There is hyperplasia of the bone marrow in this type
of the disease and evidence of an attempt at regeneration of the blood.
Hemorrhages from the skin and serous surfaces are common. The coagu-
lation and bleeding time are often prolonged. The blood platelets are
usually decreased in number, rarely increased. In the treatment of per-
nicious anemia the first essential is a correct diagnosis. Intestinal para-
sites which might account for the anemia should be excluded, and the ex-
istence of malignant neoplasms, especially carcinoma of the stomach,
should be carefully investigated. The frequent occurrence of gastric
anacidity in pernicious anemia is a point to be borne in mind, and is best
treated by the administration of full doses of hydrochloric acid. The
importance of discovering and removing any focus of infection, especially
buccal and gastro-intestinal infections, has been emphasized by William
Hunter.

The sub-group, aplastic anemia, differs from the usual type of per-
nicious anemia in that the bone marrow is aplastic, the cases run a rapid
and progressive course without remissions, hemorrhages are more com-
mon and may be very severe. The coagulation time and bleeding time are
increased. Whipple (23) investigated a case in which he found the delay
in coagulation time associated with an excess of antithrombin, the other
factors concerned in coagulation being normal. Duke (3) found great
reduction in the number of platelets in his cases.

The methods of treatment considered in this chapter may be directed
against the anemia itself or only against the hemorrhage. We will con-
sider first treatment directed against the anemia without reference to
hemorrhage. Small injections (10 to 20 c. c.) of defibrinated blood given
subcutaneously or intravenously, and repeated at intervals of a few days,
have been reported by Morawitz (12) and others. Improvement is said
to follow this procedure, the supposed effect being stimulation of the
bone marrow. If this treatment is adopted it may be desirable from the-
oretical considerations to use blood from a member of a different iso-
agglutinin group from that of the patient, with the hope that it may
prove a more efficient stimulus to the bone marrow than the introdution
of corpuscles homologous to those already in the circulation. It even
seems doubtful if further stimulation of the bone marrow is desirable,
since the mere presence of anemia forms a powerful stimulus for the hemo-
poietic organs. This is indicated by the regeneration forms present in
the blood in the usual type of pernicious anemia. In the aplastic type we
may well imagine that the stimulus is present, but that the bone marrow is
no longer capable of response, perhaps as a result of exhaustion following
overstimulation, and the protection of the hemopoietic organs from this
excessive stimulation seems a more rational form of treatment. With this
end in view one may attempt to relieve the anemia at once by transfusion.
Direct transfusion may be employed, but, for the reasons given above, the
indirect transfusion of whole blood or defibrinated blood seems preferable.
During the past three years we have employed the indirect transfusion of
defibrinated blood in a number of cases of pernicious anemia with very
encouraging results. The treatment consists in the introduction of 500
c. c. amounts of defibrinated blood at intervals of one to two weeks, thereby
relieving the anemia rather rapidly. Two, three, or four such injections
may be necessary. The interval between injections is determined by the
blood count. The introduction of 500 c. c. of blood usually increases the
count by about 500,000 red cells. Following the first, and sometimes the
second, transfusion the count may gradually fall. Counts should be made
every second or third day, and the next injection be given before the
original level is reached. It is difficult to give precise indications, but the
next injection might be given at a time when the count is still 200,000
cells in excess of the number preceding the last injection. Following the
third or fourth transfusion, in favorable cases the count does not decline,
but may show a progressive increase.

Treatment of the Hemorrhage in Pernicious Anemia.—The
hemorrhagic tendency, as well as the anemia, may be successfully com-
bated by the transfusion of large amounts of whole or defibrinated blood,
as just described, but in case other measures are employed for the treat-
ment of the anemia the hemorrhagic tendency may be treated by injections
of normal rabbit or human serum in doses of 15 c. c. intravenously or 30
c. c. subcutaneously, repeating the injections at intervals of 24 hours until
three or four injections have been given.

Leukemia.—This group of diseases is characterized by a great increase
in the leukocytes of the blood with hyperplasia of the leukoblastic tissues.
With the progress of the disease a well-marked anemia usually develops
which may become of extreme grade. Hemorrhages are not infrequent.
The bleeding may be from the skin, mucous or serous membranes. Hem-
orrhagic retinitis may occur, and profuse epistaxis may lead to a rapidly
developing anemia. The blood platelets are usually increased. This is
especially true of the myeloid form. The coagulation time in some cases
is delayed. Whipple (23) investigated a case of myeloid leukemia with
purpura and profuse epistaxis in which the blood showed an increase of
antithrombin. For the treatment of hemorrhage in leukemia one may
resort to the injection of serum, as previously described, and if the anemia
reaches a dangerous grade one may transfuse whole or defibrinated blood.
It should be remembered that this treatment is symptomatic, and prob-
ably has no direct influence on the leukemic condition which should be
treated by appropriate measures.

Spleenic Anemia.—This disease is usually associated with a marked
anemia of the secondary type which may reach an extreme degree. Hemor-
rhages are common, and may occur in the skin or from the mucous sur-
faces. Hematemesis has brought about a fatal issue in a number of
cases. For the milder grades of hemorrhage injections of serum may be
employed. In the cases with a grave anemia transfusion of whole or de-
defibrinated blood may temporarily relieve the anemia. The only curative
measure known is splenectomy. The mortality from this operation is
high, owing perhaps to the fact that many of the patients are suffering
from a severe grade of anemia, and to the further fact that the operation
is attended with grave danger of hemorrhage from the enlarged vasa
brevia which are frequently present in this disease. If the patient is
anemic at the time he presents himself for operation a preliminary trans-
fusion may do much toward lessening the risk of the operation, and in a
number of instances simultaneous transfusion has been employed at the
time of operation.
SECONDARY ANEMIA

We need consider here only the secondary anemia following hemorrhage. A discussion of the anemia associated with acute infections, intoxication, and other conditions will be considered when we come to discuss the diseases with which hemorrhage may be associated.

Acute Anemia Following Hemorrhage.—If the hemorrhage has not been excessive, and has stopped spontaneously, or has been controlled by direct means (compression, ligation, suture, etc.), little need be done beyond the ordinary upbuilding measures: rest, suitable diet, and the administration of iron. If the hemorrhage has been so severe as to endanger life the first indication is to staunch the flow of blood, if the bleeding point be accessible, and follow this immediately by transfusion of whole or defibrinated blood. If the hemorrhage cannot be checked by direct measures one may still resort to transfusion with the hope that a spontaneous cessation of the hemorrhage may take place and that the blood introduced may serve in the meantime to prevent dangerous depletion. In such cases care should be taken not to introduce enough blood to raise the pressure to a degree which would tend to cause a continuation of the hemorrhage. It is desirable to introduce just enough blood to prevent the total amount in the body falling to a dangerously low level. Indirect transfusion in such cases appears to be the method of choice, as it enables the operator to control exactly the amount of blood introduced.

Chronic Secondary Anemia.—Usually the primary indication in the treatment of the chronic secondary anemias following repeated hemorrhages is to remove the cause of the bleeding, for example, excision of gastric or duodenal ulcer, cauterization or packing in case of epistaxis, curettage for metrorrhagia, removal of hemorrhoids, or by such other measures as are appropriate. If the anemia is of an extreme grade transfusion may furnish the only hope of bringing operative procedures to a successful issue. Little good can be expected from injections of serum in such case unless the hemorrhage depends, in part at least, on a disturbance in the coagulability of the blood which may be favorably influenced by serum injections.

The chronic secondary anemia following repeated hemorrhages may reach an extreme grade even when the individual hemorrhages are small. We have recently seen two cases in which the hemoglobin was reduced to 10 per cent. One followed bleeding hemorrhoids, and the other persistent metrorrhagia. Following a transfusion of 550 c. c. defibrinated blood in the first case the hemoglobin rose to 35 per cent., where it remained about stationary for three to four weeks, further gain apparently being balanced by the continued bleeding from the hemorrhoids. A second injection of defibrinated blood raised the hemoglobin to 55 per cent., and the patient was transferred to the surgeons for operation.
The case of metrorrhagia illustrates the value of transfusion in connection with operations in the presence of a severe anemia. This patient entered the hospital on January 23, 1914, with a red count of 1,080,000 and hemoglobin of 10 per cent. Operation was decided upon, and as a preliminary measure 600 c. c. defibrinated blood was given, which raised the red count to 1,980,000 cells and the hemoglobin to 25 per cent. On the following day hysterectomy was performed by Dr. J. C. Neel, a second injection of defibrinated blood, 500 c. c., being given during the operation. The following day the blood examination showed 2,688,000 red cells and hemoglobin 40 per cent. The patient left the hospital three weeks later with 3,256,000 red cells and hemoglobin 45 per cent.

The Hemorrhagic Diseases

**Hemophilia.**—Hemophilia furnishes the example *par excellence* of a hemorrhagic disease. This condition has been the object of extensive study by numerous investigators, the results of which have been so at variance that it seems unnecessary to discuss them here. Howell (9), in his recent investigations, concludes that "the blood in this condition is deficient in prothrombin. The antithrombin may be normal or somewhat greater than normal. The characteristic peculiarity of hemophilic blood is its markedly delayed time of coagulation. This peculiarity is explained by the diminution in amount of the prothrombin which results in a relative excess of antithrombin."

Weil (21) and others have reported favorable results in the treatment of hemorrhage in this condition from the intravenous injection of fresh serum. This procedure may be useful as a prophylactic measure before minor operations which may be necessary in these patients, such as extraction of teeth, etc.

For the treatment of severe anemia following prolonged bleeding in this disease transfusion should be employed. Direct transfusion is contraindicated owing to the danger of uncontrollable hemorrhage even from the slight incision necessary in carrying out the procedure. This danger is not present when the blood is introduced by means of a needle inserted through the skin into a vein of the patient, as the elasticity of the vessel wall closes the needle puncture wound.

**Morbus Maculosus Neonatorum.**—Under the heading Hemorrhagic Diseases of the Newborn are grouped a variety of conditions which unfortunately some authors have not been careful in distinguishing from each other. Holt (7), under the title "The Hemorrhagic Disease of the Newly Born," separates a disease characterized by multiple hemorrhages of unknown etiology and not associated with syphilis or sepsis. The bleeding may come from the stomach, intestines, mouth, nose, umbilicus, conjunctivae, ears, and the skin. The condition comes on usually during the first week of life, is of brief duration and high mortality, and is self-
limited. It is not a manifestation of hemophilia, and the term hemophilia neonatorum should not be applied to it: Osler (19) draws attention to the fact that not every case of melena neonatorum belongs in this category, as ulcer of the esophagus, stomach, and duodenum may give rise to the presence of blood in the stools and, in some instances, the blood which appears in the stools may even be drawn from the breast of the mother. In the study of this group of cases great care should be exercised to determine the exact nature of the condition present and to designate it by a name which will not lead to confusion. Instead of designating one disease by a name which is descriptive of the whole group, it would perhaps be better to employ the less usual, and not adequately descriptive, but more individual name, morbus maculosus neonatorum. We have but little data upon which to draw conclusions as to the underlying cause of the hemorrhagic tendency in this condition.

Whipple (23, 24) investigated two cases which seem to fall in the above category, and although in one the mother gave a positive Wassermann reaction, the placenta was normal and no evidence of syphilis was found at the autopsy of the infant. The blood of both cases showed a markedly delayed coagulation time, and there was complete absence of prothrombin.

The results of the treatment of this disease by transfusion and by serum injections have been most gratifying. Cures have been reported in a large number of cases. If the amount of blood lost has been large, and the resulting condition of the infant is critical, immediate transfusion of whole or defibrinated blood is indicated. The amount of blood introduced should probably not exceed 200 c. c. If the hemorrhage has not led to a severe anemia, and the condition of the child is fairly good, satisfactory results may usually be obtained by the intravenous injection of fresh rabbit or human serum in 10 c. c. doses or the subcutaneous injection of 15 to 20 c. c. amounts. The disease is of short duration and self-limited, progressing to death or recovery in a few days. The mortality in 709 cases collected by Townsend was 79 per cent. Prompt and vigorous treatment is demanded. The serum injections should be repeated at intervals of three to six hours, and if the bleeding continues transfusion should be resorted to before the patient's condition becomes too serious. Schloss and Commiskey (20) have reported good results from the subcutaneous injection of whole blood in 10 c. c. amounts. In a case which recently came under our observation the bleeding was apparently uninfluenced by this procedure, and twelve hours later there was no evidence that the blood injected had been absorbed. Two subcutaneous injections of pure thrombin prepared according to Howell's method (8) were then given by Dr. Goodpasture. The hemorrhage ceased after the second injection and the patient left the hospital a week or ten days later in satisfactory condition.
Arthritic Purpura.—Under this heading two types of purpura are described, purpura simplex and purpura rheumatica. We have never seen any good results attend the use of serum injections in these conditions, although there are a number of favorable reports in the literature. The method seems worthy of a further trial in these cases.

Purpura Hæmorrhagica.—In addition to purpura there may be excessive hemorrhages from the mucous membranes, epistaxis, hematemesis, and hemoptysis, leading to a profound anemia and, in some instances, a fatal termination. Duke (3) has reported a great reduction in the number of platelets in the cases studied by him. Howell (9) found no disturbance of the prothrombin-antithrombin balance in his cases, but the number of cases studied is too small for generalization. There are many reports of prompt and completely successful results from the use of fresh human serum and normal rabbit serum in the treatment of these cases. If this measure fails, and the hemorrhage has reached alarming proportions, transfusion of whole or defibrinated blood should be performed.

Essential Hematuria.—The etiology of this condition is entirely unknown, and we have been unable to find any data upon the condition of the blood in this disease. Injections of normal serum may be tried, and if the anemia has reached a dangerous degree transfusion may be employed.

Diseases with Which Hemorrhage May Be Associated

It would be useless to go through the long list of diseases given under this heading and attempt to point out the conditions in which the methods of treatment considered in this chapter might be applicable. In a majority of cases the necessity for the introduction of serum or blood would not arise, and in those cases where it did arise one should attempt to meet the indications of the individual case. In the following pages only a few of the diseases mentioned will be discussed.

Typhoid Fever.—In this disease the coagulation time of the blood may be shorter or longer than normal, corresponding perhaps to the occurrence of thrombosis in some cases and to hemorrhage in others. There can be little doubt, we think, that the bleeding in many cases of typhoid fever is accompanied by a disturbance in the balance of the factors influencing coagulation. We have treated a number of cases of typhoid hemorrhage by intravenous injection of serum, and while realizing the difficulty of drawing conclusions from anything less than an extensive series of cases, we feel that the results warrant a further trial of the method. In cases where the hemorrhage has been profound we have not hesitated to resort to the indirect transfusion of defibrinated blood.

Tuberculosis.—In the chronic pulmonary form of the disease the hemorrhage is most frequently due to the erosion of vessels or the rupture of small aneurisms in the lungs. It seems unlikely that the bleeding in such
cases would be influenced by any of the measures under consideration here. Cases of tuberculosis occur, however, in which there appears to be a disturbance of the coagulability of the blood. Duke (3) reports a case of tuberculosis associated with purpura which showed a prolonged bleeding time and low platelet count. He also reported a case of tuberculosis associated with epistaxis in which the bleeding time was prolonged and the platelets reduced. Whipple (23) reports a case of miliary tuberculosis with profuse epistaxis in which the examination of the blood showed a low fibrinogen content. We have no observations upon the results of serum treatment of hemorrhage in tuberculosis, but the method seems worthy of trial.

Pellagra.—Transfusion has been recommended for the treatment of this disease. The series of cases thus far reported are too few to permit of drawing conclusions.

Gastric and Duodenal Ulcers.—The hemorrhage in this condition is dependent upon the erosion of vessels by the ulcer, and in the acute cases there is probably no disturbance in the coagulability of the blood. If the bleeding has been copious, resulting in the production of an acute anemia, the transfusion of whole or defibrinated blood is indicated. The blood pressure should be observed during the operation, and the amount of blood introduced should not be large enough to increase the pressure above normal.

A recent experience may be reported in this connection. The patient, a very robust iron worker, without any previous symptoms pointing to gastric ulcer, suffered three profuse hematemeses on the night of March 3, 1914. Following the third hematemesis he fell unconscious to the floor. The next day he was brought to the hospital in a weakened condition, and on admission the blood examination showed:

<table>
<thead>
<tr>
<th>R. B. C.</th>
<th>W. B. C.</th>
<th>Hb.</th>
</tr>
</thead>
<tbody>
<tr>
<td>4,228,000</td>
<td>21,800</td>
<td>66 per cent.</td>
</tr>
</tbody>
</table>

The patient continued to vomit copious amounts of blood on March 4 and 5, and the feces contained much dark blood. The blood count on the evening of the latter day had reached the following figures:

<table>
<thead>
<tr>
<th>R. B. C.</th>
<th>W. B. C.</th>
<th>Hb.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,080,000</td>
<td>15,600</td>
<td>30 per cent.</td>
</tr>
</tbody>
</table>

The blood pressure ranged between 60 and 80 mm. of mercury. At this time 18 c. c. of fresh rabbit serum were given intravenously. Following this injection there was but one hematemesis. This occurred the next day, and while the amount was only 50 c. c. it was followed by a syncopeal attack, and tarry stools continued to be passed. On March 6 the red cells were 1,672,000 and the hemoglobin 25 per cent., and later in the day fell to 22 per cent. The respirations were sighing in character, and the patient semi-comatose. The condition seemed so critical that it
was decided to transfuse. A donor was selected of the same iso-agglutinin group as the patient, and 230 c. c. defibrinated blood were introduced. The blood pressure was observed at intervals of a few minutes during the procedure, and the transfusion discontinued when the pressure reached 118 mm. of mercury, as it was feared that a further increase of pressure might start the hemorrhage again. After six or eight hours the pressure had fallen to 80, and a second injection, 475 c. c. defibrinated blood, was given. This was followed by a striking improvement in the patient's condition. The blood count showed on March 7, R. B. C. 3,116,000; Hb. 40 per cent., and on March 10, R. B. C. 3,200,000; Hb. 44 per cent., since which time the convalescence has progressed satisfactorily. Although the diagnosis in this case remains in some doubt, the indication for transfusion seemed clear enough, and the results may, with reasonable confidence, be referred to this measure.

Jaundice.—Although the hemorrhagic tendency in many cases of jaundice has long been recognized, no satisfactory explanation has been brought forward to account for it. Whipple and King (25) have suggested that the bile pigments have combined with the calcium salts of the blood in such a way as to render them incapable of playing their part in the formation of thrombin.

Morawitz and Bierich (13) maintain that, although the bile and the gallic acid salts are capable of inhibiting coagulation, that the concentration necessary for this action is never reached in the circulating blood. The coagulation and bleeding time may be delayed in some cases of jaundice and not in others. Whipple (24) has found that in cases of jaundice associated with liver disease there may be a reduction of the fibrinogen of the blood.

The danger of hemorrhage following operation on jaundiced patients is well known, and further study of the blood in this condition may furnish a means of determining in advance those cases in which bleeding may prove a troublesome feature and those in which no danger may be expected from this source.

At present a delayed coagulation and bleeding time are usually taken as an indication of danger. In such cases prophylactic injections of serum may be tried, and if the coagulation and bleeding time return to normal operation may be performed with little fear of hemorrhage. If hemorrhage occurs spontaneously or following operation in a jaundiced patient serum injections may be employed, and there are numerous reports of favorable results attending their use. Transfusion may be necessary in the graver cases of hemorrhage. Cases with deficient fibrinogen would probably be influenced favorably only by the introduction of whole blood.

Diseases of the Liver.—Whipple (24) and others have found a deficiency of fibrinogen in a variety of diseases of the liver with and without jaundice. It is unlikely that the primary disease in any of these cases could be influenced by the methods here considered. In the case of hemorrhage it seems unlikely that injections of serum or of defibrinated blood
REFERENCES

would be of value, since neither of these agents contain fibrinogen. It would be more rational in such cases to introduce whole blood.

**Metrorrhagia.**—Favorable results have been reported by Busse (2) from the use of serum injections in metrorrhagia occurring under various conditions.

REFERENCES

6. ———. Unpublished work.
19. Osler. The Principles and Practice of Medicine, 8th Ed.
25. —— and King, J. T. Unpublished work.
CHAPTER XXXVIII

THE USE OF SERA AND VACCINES IN OBSTETRICS AND GYNECOLOGY

N. SPROAT HEANEY

In the past it has been particularly true of this department of medicine that new cures were heralded with great acclaim, widely adopted, and as rapidly forgotten. The natural reparative powers of the body, the self-limitation of certain affections, and the natural tendency in many diseases to spontaneous cure were not properly understood, and the return of a patient to health was attributed to the particular therapeutic measure employed. As a rule most of the principles of treatment were drastic, and lost sight of the tenet that the first qualification of a cure is that it shall do no harm; so that quite frequently, aside from those measures adopted to increase the general tone of the patient, the therapeusis did the patient more harm than good. The truth of this assertion may be best appreciated by a study of the history of the treatment of puerperal infection. The literature is crowded with specific cures. Intravenous injections of formalin, mercury, and other potent poisons; intruterine douches of carbolic acid and of various other medicaments of equal capacity for harm; curettage as a routine treatment, and even major operative procedures, have from time to time been advocated as the principal hope in the treatment of this dreaded affection. Contrast our treatment in the larger clinics to-day. Fresh air, an abundance of good food, and rest in bed are the essentials of the modern treatment of puerperal sepsis. Often all that is done beyond this is directly harmful. The eradication of the disease itself is as yet beyond our power; all we can do at the present is to increase the natural defenses of the body.

It requires time and the observation of a large number of cases to judge the value of the effects of a new procedure; only in isolated instances can judgment be based upon the results obtained in a few cases or by a single observer.
SERUM, DEFRIBRINATED BLOOD, AND WHOLE BLOOD IN THE TREATMENT OF HEMORRHAGIC DISEASES

The results obtained in the treatment of certain hemorrhagic affections by the use of serum, defibrinated blood, or whole blood, are often extremely gratifying. It appears that the bleeding may be due to the excess or deficiency of certain substances present normally in balanced amounts.

In order to understand the pathology of bleeding it is necessary that something be known of the physiology of clot formation. Howell (4) believes that a small amount of antithrombin is present in the normal plasma, and is sufficient to bind the prothrombin. Thromboplastin is set free by cell injury, and neutralizes the antithrombin; this releases the prothrombin, which at once combines with calcium to form thrombin. The free thrombin coagulates the fibrinogen, and the normal clot is produced. Some of the agents necessary to the normal production of clotting may be present in abnormal amounts, and thus a delayed clotting time result. Whipple (4), in a study of the various factors in abnormal clotting, ascertained that the balance existing between antithrombin and prothrombin is variable, and should be studied before treatment is administered. The hemorrhage may be due to an excess of antithrombin, or to a decrease in the prothrombin. It is believed that antithrombin is produced in large part by the liver, and in some diseased conditions may be produced in excess.

On theoretical grounds it does not appear reasonable in a case where antithrombin is in excess to introduce into the circulation defibrinated blood which is itself rich in antithrombin. When antithrombin is in excess the hemorrhage should be treated by a direct transfusion of whole blood. Whipple reports a case in which there was an excess of antithrombin where the use of defibrinated blood seemed to accentuate the hemorrhage. In prothrombin deficiency, on the other hand, serum makes up the deficiency, and is efficacious, though defibrinated or whole blood may be used. It is thus seen that the choice between serum, defibrinated blood, and transfusion in a given case of hemorrhage may not be a matter of indifference. However, our knowledge concerning these hemorrhagic affections is not extensive, and it is impossible always to explain the effects of successful therapy. For some time it has been known that the serum of another individual or animal, when injected into a person suffering from a hemorrhagic affection, is capable, in many instances, of stopping the bleeding.

When the patient is in imminent danger of death from exsanguination the indication is for a direct transfusion; but when there are repeated small losses of blood, from which the system can recover providing the
bleeding is fairly promptly checked, either defibrinated blood or serum may be effective, unless the cause lies in antithrombin excess, in which event Whipple believes that their use is contraindicated.

In some cases normal horse serum seems to be as effectual as human serum so far as the arrest of hemorrhage is concerned, yet its employment is so intimately associated with the dangers of anaphylaxis, the ultimate consequences of which we are only beginning to appreciate, that its use should be restricted to cases where a satisfactory human serum is not obtainable, or where the initial dose cannot be delayed until the donor can be sufficiently investigated. In such an instance a single dose of horse serum may be given, to be followed later by human serum.

The usual dose of serum is 20 to 30 c. c. at an injection, and this amount is to be repeated two to six times in the 24 hours. In cases suited to this form of treatment bleeding usually ceases within that time. Normal horse serum is procurable on the market in the same form as the various protective sera. Antitubercular serum may be used if normal serum is not available.

The Selection of a Donor.—As much care should be exercised in the selection of a donor for serum as is usual for direct transfusion. A careful physical examination, a searching history, and a negative Wassermann reaction are prerequisites. The only permissible deviation from this rule is when the blood of one of the parents is to be used for the treatment of their newborn child. Not only should all the evidence be negative, but the history should be above all suspicion. No case is so urgent that a questionable donor should be taken, even if all tests are negative. The taking of a donor solely because she is the mother of apparently healthy offspring cannot be too severely condemned. It is true the chances are small that such a selection would be followed by disaster, but harm has occurred so frequently from this sort of "reasoning" in the choice of wet nurses that no condemnation is severe enough to characterize the one who disregards modern methods of guarding against the possibilities of transmitting syphilis by his random choice of a donor of blood or of some of its components.

The blood may be obtained as in venesection, allowing 200 to 300 c. c. to collect in a sterile flask or beaker, but it is best procured by a more careful technique. A constrictor is placed tightly about the biceps, and the region of the cubital vein surgically cleansed. The vein is entered with a needle and the blood withdrawn into a sterile container. The constriction is then removed, the syringe drawn out, and the puncture point compressed for a moment and sealed with collodion. The donor experiences no unpleasant sensation aside from the prick of the needle.

If defibrinated blood is desired the blood is immediately beaten with a sterile rod or stick, or shaken in a flask with sterile glass beads before clotting has time to occur. The fibrin separates, leaving the blood ready
for use. If serum is wished the vessel is put aside at room temperature. Usually within an hour enough serum exudes for the first injection. Sakoguchi (3) advises leaving a sterile stick or folded wire of sufficient size to protrude above the surface of the blood; the blood will clot about this object, and can be removed, leaving clear serum. Since serum rapidly deteriorates, the supply should be kept upon ice and great care used to prevent contamination. If the serum becomes cloudy its use sometimes produces slight fever. It should not be given when kept longer than 48 hours, as the complement-content rapidly lessens on standing.

**Dosage of Serum.**—Failure frequently results from insufficient dosage. Thirty cubic centimeters are to be given at a dose, and this dose is repeated, according to the necessities of the case, twice daily, or every four hours, until the desired result is obtained or failure is demonstrated. Usually the treatment is effectual within 24 hours, and may be discontinued within 48 hours. The injections are made with a syringe that has been sterilized by boiling. The injections are given subcutaneously or intramuscularly into the tissues of the thigh or back. The intravenous method of giving defibrinated blood should be practiced only by those who have had experience, because of the danger of embolism.

**Hemorrhagic Disease of the Newborn**

Hemorrhagic disease of the newborn appears as a menace to the life of the child in the early puerperium, usually within the first week, rarely after the seventh day. Usually insidious in its onset, it is only occasionally that any considerable amount of blood is lost as the first symptom. The diaper may show a tarry stool of digested blood, or a bright spot of blood may show in a normal bowel movement. Oozing may occur from the umbilical stump, from a slight abrasion produced at birth, from a circumcision wound, or from the vagina, or other mucous surface. The child may vomit blood, or there may occur subcutaneous hemorrhages with formation of hematoma. The child may bleed from a number of places as the affection increases in severity. If suitable treatment is not promptly instituted the babe gradually grows weaker and dies of exsanguination. No more gratifying results are secured in any therapeutic field than are obtained by the use of serum in this disease. Usually the physician delays to see whether the bleeding may not be stopped by some of the familiar styptics, and only turns to the use of serum in extreme cases. If the serum of the mother or of another absolutely safe individual is used, and if sepsis is maintained, the treatment is devoid of danger. Early administration and frequent repetition of the serum in sufficient dosage will give the desired result in 100 per cent. of the cases. (Welch, 8.) The essential condition appears to be, according to Whipple's investigations, an insufficiency of prothrombin, and this lack is remedied by
the giving of normal serum. Besides the danger of anaphylaxis, horse serum, if given in large amount, is said occasionally to increase the bleeding.

Some have advised the giving of defibrinated blood instead of plain serum. Ehrlich has shown that the presence of the red-cell elements causes the elaboration of a hemolytic isolyisin for its digestion, and this act requires work of the already greatly overburdened constitution. Serum contains all the elements necessary for the hemostasis, and once the hemorrhage is stopped the child will rapidly regain its hemoglobin content.

Serum will not save a child on the verge of death from exsanguination; the only remedy in such a case is direct transfusion. The child should be prevented from such an extremity by the early use of serum, but in case treatment is instituted late the only hope may be in transfusion. Usually this requires the services of an expert, but with the device perfected by Curtis and David (66) the operation is easily performed. The smallness of the vessels and their collapsed state increase the attendant difficulties. There is, of course, the possible danger of hemolysis, produced by the entrance of the foreign blood, and also the chance of the production of emboli. However, with proper precautions, these accidents are infrequent, while the results of the operation are striking. Transfusion is not the treatment of choice in hemorrhagic disease of the newborn. If the case is not an advanced one the early use of normal human serum will be as successful as transfusion, and is devoid of many of its difficulties and dangers.

**SERUM IN THE TREATMENT OF UTERINE BLEEDING**

Occasionally one meets with individuals in whom, in the absence of accountable local pathology, the menstrual periods are profuse and debilitating. Quite frequently the subjects are young girls in whom the underlying cause is, perhaps, a disturbance in the internal secretions, and yet all attempts at amelioration of the condition may fail. Many are subjected to curettage with the idea that there may be an abnormal endometrium. If, upon curettage and examination of the scrapings, no pathology is found the bleeding will probably continue without change, or, at best, improvement will be only transitory. Good results have been obtained in this class of cases by the injection of human serum, defibrinated blood, or normal horse serum. A single dose of one or two ounces of serum or defibrinated blood is often followed by permanent relief; occasionally the treatment must be repeated in three or four months.

Before this treatment is instituted the physician must absolutely eliminate the presence of pelvic disease, the delay of suitable treatment of which would be detrimental; and he should especially bear in mind that there is no age limit for cancer of the womb, since it has been found in girls under twenty years of age.
In addition to the hemostatic effect, treatment by serum or blood seems to be directly stimulating to the production of red cells. Zubrzycki and Wolfsgruber (10) report that, in women suffering from carcinoma of the cervix, they succeeded by the use of 140 c. c. of defibrinated blood in raising the hemoglobin from 25 to 35 per cent., and the reds from 1,500,000 to 3,800,000 during the course of four weeks.

W. Meyer (2) has used normal human serum in the prophylaxis and treatment of the parenchymatous hemorrhage occurring after operations upon subjects suffering from icterus and hemophilia with encouraging results. He gave one to two ounces three times daily for two days preceding, and for at least two days following, operation.

**Human Serum in the Induction of Labor**

The essential factor that brings about labor has not as yet been satisfactorily determined. That it is some substance that gains entrance to the blood and thus brings about uterine contractions, and that this substance is probably of the nature of a hormone, has long been believed. The observations made on the Blazek twins, the behavior of animals joined together in symbiosis, the results of animal transfusion, show that there is something, previously absent, which appears in the blood of the pregnant woman at the time of labor. Heyde (12) thought that he might bring on labor prematurely by the injection of serum obtained from women in labor. He was enabled to bring about uterine contractions hereby, but did not succeed in inducing labor. Thinking that the necessary substance was fetal in origin, and consequently present in the mother's serum in such dilution as not to be demonstrable, he tried the same experiments, using the serum obtained from the blood coming from the cord after the release of the child. Upon the injection of this serum he obtained undeniable effects.

Rongy (11) has duplicated these results in 19 women. In six women who were from 10 to 18 days from term one or more injections induced labor pains which terminated in birth. In seven patients the results were entirely negative, while in the remaining six the contractions were transitory. He reports that frequently after the injections there were chills, nausea, and vomiting, and, sometimes, precordial pain and oppression. This very interesting work is purely experimental and has not been adopted in active therapeutics.

**Normal Serum in the Treatment of the Intoxications of Pregnancy**

Schmorl and Veit have described the presence of placental elements in the free circulation of the pregnant woman. Considerable proof is at
TREATMENT OF HEMORRHAGIC DISEASES

hand that an increase in the digestive power of serum occurs in pregnancy, and that these digestive ferments are elaborated for the purpose of freeing the blood stream of placental elements. It is thus supposed that there are produced in the blood stream of the normal pregnant woman protective bodies in sufficient amount, while in those women who come under the classification of intoxications of pregnancy these bodies are insufficient to overcome the noxious effects of the placental products. The attempt was therefore made to relieve certain of the intoxications of pregnancy by the injection of the serum of normal pregnant women.

Reports have been published dealing with this usage in not only eclampsia and the pernicious vomiting of pregnancy, but especially in the dermatoses. Richard Freund (14) reviews the results of the serum treatment of the intoxications of pregnancy in the German literature, and finds that of the dermatoses, under which are included cases of herpes gestationes, urticaria, pruritus, lichen urticatus, general prurigo, and pemphigus-like dermatitis, twelve cases were treated. Some found complete relief immediately upon the injection of 10 to 25 c. c. of serum, while others required a repetition of the dose. When the stubborn nature of these affections is considered these results are encouraging. Of the cases suffering from hyperemesis there were five; in two cases there was immediate benefit; in two marked cases repeated injections failed to give relief, and pregnancy had to be terminated; in one case vomiting ceased six days after treatment. The results in pernicious vomiting are such as may be obtained from any therapy, no matter what its nature. Other cases are reported where the serum seemed to stop the vomiting, but the women later aborted. In such an event one must not overlook the possibility that the cessation of vomiting coincided with the death of the ovum. Serum from pregnant women, combined with venesection, was tried in six cases of eclampsia, with results that could not be credited either for or against the treatment.

Freund shows that the effect of this treatment apparently is not dependent upon the presence of protective bodies in the serum of normal pregnant women, since just as good results were obtained when normal horse serum was used. He believes that the results of its use are ascribable to the calcium content of the serum rather than to any specific substance, since, in 15 cases of dermatoses of pregnancy treated with injections of 150 to 200 c. c. of Ringer’s solution, the eruptions very promptly disappeared. Rissman (21) was also able to effect a very prompt and permanent cure in three dermatoses of pregnancy by the injection of 185 c. c. of Ringer’s solution, the symptoms beginning to recede within a few hours of the injection. Since this medication is freer from harmful possibilities, it had better be tried in these resistant intoxications before submitting the patient to the administration of serum.

Vinnay (22) reported a case of hyperemesis gravidarum which he
treated by direct transfusion of blood from a normal pregnant woman. Vomiting almost completely ceased after the transfusion, though she developed a mild icterus and aborted two months later.

THE USE OF ANTISTREPTOCOCCUS SERUM

When antistreptococcus serum was first introduced the profession was very hopeful that it might cure the many cases of streptococcus infections which had so consistently resisted all attempts at treatment in a high percentage of cases. Especially in puerperal infection the prospects seemed bright of ridding that malady of its terrors. Therapeutic results obtained with the serum did not demonstrate its efficiency, and after a short period of popularity serum was much less used.

More recent experimental work by Weaver and Tunnicliff (23-28) shows that in animals the injection of antistreptococcus serum is followed by an increased phagocytic power of the leukocytes of brief duration, and an increased opsonic power for streptococi for a period of about ten days, and that animals can be protected by serum against doses of streptococi that are uniformly fatal to control animals. Their attempts, however, to treat well-established cases of infection were not successful.

These workers draw attention to the facts that antistreptococcus sera rapidly lose their opsonic power, and that one is not certain of procuring an active serum. If the serum is to be used the dosage must be large, 30 to 100 c. c. Weaver further advises that if the serum is to be used in a curative way it should be given early, and if one wishes to obtain a rapid effect it should be administered intravenously, or, when this is impossible, intramuscularly, though by this route the effect is somewhat slower. The subcutaneous administration apparently can show no effects before about 24 hours. The benefit of the medication should be shown by a prompt fall in the temperature, an increase in the opsonic index, a reduction of the leukocytosis, and by the clinical improvement of the patient's condition. When the improvement comes to a standstill, when the leukocytes again increase, or the opsonic index falls, a repetition of the dose is indicated.

In view of the experimental results, the use of antistreptococcus serum is indicated early in the course of an infection, especially when the possible dangers from its use are minimal compared to the possibilities of benefit. The results obtained by the use of serum in streptococcus puerperal infection, however, require careful interpretation and large clinical experience, for the reason that infections by the streptococcus show wide and sudden variations of the clinical picture independently of therapeutic measures. The interpretation of results obtained when the serum is given early in the infection requires special care. In this stage we see many quick returns to the normal, no matter what the therapy. No conclusion
based upon an isolated case or upon a small number of cases is allowable.

The largest field of usefulness, judging from the experimental data, is in the prophylactic treatment of streptococcus infections. The high mortality rate in the operation for the radical cure of cancer of the uterus is due largely to the peritonitis engendered by the entrance of streptococci into the peritoneal cavity through the opening of the infected vagina, or by the rupture of infected lymph nodes during the operation. To minimize the danger of a post-operative peritonitis it has been advised to take a culture from the vagina in such cases, and when streptococci are demonstrated to immunize the patient by the administration of an autogenous vaccine and antistreptococcus serum. The same may be done when a radical operation is to be performed for the removal of a vaginal or abdominal fistula which yields streptococci, no matter if the patient has been temperature-free for a considerable time. During operations for the removal of pus tubes rupture of a tube is frequent in spite of the exercise of extreme care. In acute cases the pus often contains streptococci, and for this reason clinicians avoid by all safe means operations on pus tubes during the acute stage. In chronic tubes the pus is usually sterile, but occasionally it contains streptococci, which may usher in a fatal peritonitis. It has been suggested that, whenever pus escapes during an operation for pus tubes, a smear and a culture should be made, and in case streptococci are found an early prophylactic dose of antistreptococcus serum should be given.

That the patient recovers after the administration of the serum in such a contingency is not direct evidence of the effect of the serum, however, since patients frequently recover with little disturbance where streptococci have been found in the pus escaping from a tube during operation. In this connection it must be remembered that the streptococcus is frequently the secondary invader of a tuberculous or gonorrheal tube, so that the clean-cut clinical history, or typical appearance of the pathology, does not prevent the cautious man from minutely examining spilled pus.

In obstetrical cases that have been dirtily handled, or where for some other reason it seems probable that the patient will develop a puerperal infection, a prophylactic dose of antistreptococcus serum may be given. Of course it is not certain that the infection, should it occur, will be due to the invasion of streptococci, yet the chances are great that this organism will be the cause of the infection.

**THE TREATMENT OF Puerperal Infection by Vaccines**

Under puerperal infection we include any infection of the genitalia which manifests itself by the appearance of fever during the puerperium,
SERA AND VACCINES IN OBSTETRICS

no matter how brief the duration, what the infecting organism is, or how limited or extensive the area of infection. The patient may seem extremely ill, and within a few hours be temperature-free, or, with the same initial symptoms, the patient may be ill for weeks. A perineal tear may be the only seat of the infection, or the patient may have every pelvic organ, and even distant organs, involved. There is no criterion by which to prognosticate the outcome in a given case, and especially is there no way of judging the intensity of the disease in reported cases. Organisms may be cultivated from the blood of a case that recovers, while repeated attempts may yield sterile cultures in a fatal case. The results of cultural examination of the lochia allow of no prognostic conclusions. No affection holds so many surprises. A patient on the third day of the puerperium may have a violent chill with high temperature, and the next day return to the normal course of convalescence. Another patient may have fever for days and then suddenly begin to improve for no accountable reason. Because of these facts the experienced physician hesitates to ascribe a recovery to a single therapeutic measure. It is almost impossible to form any conclusions as to the effect of therapy in this affection, because of the great variations mentioned above. The clinical results must be uniformly striking in large series of accurately reported cases, or reliable laboratory methods must show undeniable evidences of benefit, before men of experience will be willing to accord to any advocated therapy specific power.

Thus far the advocates of vaccine therapy in puerperal sepsis have failed to produce these necessary proofs. On the contrary, there is every evidence to support the belief that vaccines employed in cases suffering from sepsis may be directly harmful. In the laboratory, where exact conditions can be produced in experimental and control animals, vaccines given in sepsis are either without effect or are directly detrimental. The use of sensitized vaccines may yield better results.

When, however, the fever has receded and a localized inflammation is left vaccines may be employed. Abscesses that have been drained and, though producing no fever, refuse to heal frequently react promptly to vaccine therapy. With this possibility in mind it is advisable to make cultures of all abscesses at the time of operation, for the attempt to get cultures later may be difficult or impossible.

Infections of the puerperal breast are frequently chronic. The original abscess may be slow to heal, or multiple foci may appear, producing little or no fever. Breast abscesses, in a considerable percentage of cases, are due to the staphylococcus.

Whatever the organ involved the causative organism must be identified before success with vaccines can be expected. Here, as elsewhere, the percentage of cures is increased if the vaccine is made from the organisms infecting the patient.
VACCINE TREATMENT OF GONORRHEAL INFECTIONS OF THE FEMALE GENITALIA

In order to interpret the results of the vaccine treatment of gonorrheal infections in women certain of the facts concerning the peculiar pathology must be borne in mind. Unlike the fresh infection in man, which is usually associated with more or less discomfort that forces the subject of the infection to seek relief, gonorrhea in women, unless accompanied by urethritis, very frequently runs its complete course without producing symptoms suggesting its presence. The usual female sufferer from gonorrhea presents herself to the physician because of the late manifestations of the disease, chronic endocervicitis, endometritis, or because of a bartholinitis or pelvic inflammation. Smears taken from the accessible surfaces at this time may show no diplococci, either because they are so diminished in number as to escape detection, or because they have disappeared and other invaders have taken their place. More reliable than the examination of smears is the investigation by means of cultural methods. However, even with good technique a negative culture does not acquit the case of suspicion, because the organisms may be located in inaccessible crypts.

The gynecologist is frequently confronted with a patient whose clinical history is definite, and in whom every fact points directly to the conclusion that the woman is suffering from the consequences of a gonorrheal infection. The husband tells of specific urethritis immediately preceding his wife's illness, the onset of her sickness is typical in every detail, she is treated medically as an undoubted case of gonorrheal infection, and yet, when operated upon because of invaliding pus tubes, the tissues and pus submitted to bacteriological investigation reveal no gonococci. The case is undoubtedly gonorrheal in origin, but other organisms, the colon bacillus, the staphylococcus, the streptococcus, or anaerobic organisms, are now present, and the original organism has disappeared from the tissues. The more remote the original infection the less the chance of finding the gonococcus. In the presence of fairly large collections of pus there may be complete absence of all organisms. Thus Wertheim, in an examination of 116 pus tubes, without respect to their duration, found that 72 were sterile, while Martin found sterile pus in 73 out of 109, and Menge in 68 out of 106 specimens (41). Improved cultural methods probably will show a smaller percentage of sterile examinations, but the fact remains that pus tubes are frequently sterile.

Granting that the gonococcus has been found in the smears or cultures from the cervix, is this proof that the swellings in the pelvis are due to the gonococcus? It is strong evidence, but not conclusive, as those who operate upon such cases soon learn. The cervicitis may be recent, and the tubal disease an old tuberculosis, the remnants of a post-abortive or
SERA AND VACCINES IN OBSTETRICS

puerperal infection, or, even in the presence of the strongest circumstantial evidence, the swellings may not be inflammatory at all. If, under such circumstances, the cervical inflammation is the condition that is to be treated, then the use of gonococcus vaccine may be considered, but if the patient is to be treated for the pelvic swellings the evidence that she is suffering from an existing gonorrheal infection of the appendages must be established upon reliable data. If the patient has a gonorrheal urethritis, and at the same time a tuberculous salpingitis, no one can expect to rid the patient of her tubal symptoms by the administration of a gonococcus vaccine.

The internist does not administer vaccines to a case of arthritis without an attempt to determine the etiology by searching for the causative organism in the articular fluid or in the glands draining the joint. This cultural evidence failing, he may give a vaccine upon the basis of other evidence, but in so doing he feels that his chances of success are certainly decreased. In the same way direct evidence should be sought as to the organism existing in the tube at the time that vaccine therapy is instituted if one expects beneficial results. This evidence may be gained by vaginal incision, or by the use of the exploratory needle. Vaginal incision may be directly curative in itself, but the vaccine should be made from the pus obtained and held in readiness for later use. The exploratory needle is so slender that it may often be used for securing pus in cases that are not suited to vaginal drainage. When the evidence is strong that the gonococcus is the organism in question, yet reliable proof is not obtainable, the case may be treated tentatively with gonococcus vaccine, provided it is clinically ready for vaccine treatment. Success or failure cannot be definitely credited either for or against vaccine therapy in such cases.

There would not be so much controversy to-day concerning the success of the vaccine treatment of gonorrheal affections in women if clinicians should definitely determine in an incontrovertible way that the diseases of the appendages that they are attempting to treat are due to active gonococcal infection. The simple statement that the cases treated are suffering with gonorrheal tubes is not sufficient. Etiologically the chance of the correctness of this diagnosis is great, since Wertheim and Menge (54) are the sponsors for the statement that 82 per cent. of all pus tubes are gonorrheal in origin.

We will grant that the patient is suffering from a gonorrheal infection. Before we treat her in any way at all we must know what chances she has of recovery without medication if we are to be able to judge competently of the effects of therapy. As stated, the infection may be limited to the cervix, to the urethra, or to the entire lower genital tract without ascension and recover completely without attracting any particular attention. Even after the tubes are involved the symptoms may be slight.
Cases are occasionally operated upon for sterility in the absence of any history of previous illness, and evidences of an acknowledged gonorrhea of the husband found in the closed tubes of the wife. Even after a violent attack of salpingitis one may see a rapid shrinkage in size of the tube and a return to normal function, as demonstrated by subsequent pregnancies. In general, however, the effects of gonorrheal infections of the Fallopian tubes are of long duration, and are associated with an amount of pain that is disproportionate to the other clinical symptoms.

A study of available statistics shows that the usual non-operative treatment of gonorrheal infections of the tube results in a symptomatic cure in from 50 to 90 per cent. of the cases, or an average of about 70 per cent. The objective cures also vary greatly. Fromme and Collman (40) had 30 per cent. of recoveries, Forsner (37) had 20 per cent., while Cukor (35) reports as high as 52 per cent. of satisfactory results. Probably a conservative average of complete objective cures would be about 30 per cent. of the cases. When studying the results of vaccine therapy of gonorrheal infections in women no conclusions can be based upon isolated cases, but the results of its use must be compared with those obtained by the usual conservative methods of treatment.

Heinsius (42) treated 10 cases of probable or proven gonorrhea in women with vaccines. Eight tubal cases gave good results, the duration of treatment averaging four weeks. A case of cervical gonorrhea was improved. The only instance in which treatment was without influence was one of subacute cystitis.

Fromme and Collman (40) treated a number of urethral, uterine, and cervical infections, in which the gonococcus was identified, without the slightest result. In fact, they saw Bartholinitis and ascension of the infection occur in spite of treatment. In 45 cases of pyosalpinx, in which they either isolated the organism or obtained an unquestionable history, they secured good results. They noticed regularly a subsidence of the subjective symptoms. In 10 of the 45 tubal cases a complete objective and subjective cure was secured, while 19 were subjectively cured and objectively markedly improved (decreased size of swellings, etc.). Six cases received only slight benefit, and 10 were not benefited. They therefore obtained 64 per cent. of satisfactory results. Regarding an objective cure they remark that one cannot demand a complete restoration to normal from any treatment in old cicatricial tubes in which extensive connective tissue changes have occurred.

Schindler (48) says that he has not been able to influence cases of mucous membrane infection, but has obtained notable results by the use of vaccines in gonorrheal tubes.

Slingenberg (49) is guarded concerning his experience with cases of vulvovaginitis, but thinks that cervical and uterine infections are favor-
ably influenced by vaccines, that the bleeding lessens, and the discharge disappears. He does not give his results in detail.

Heynemann (44) treated five cases of gonorrheal tubes with gonococcal vaccine without appreciable results.

Friedländer (38) saw complete restoration to normal in three cases of recent tubal infection after four weeks of vaccine therapy.

Dembskaja (36) treated 200 women having various lesions, and after two years of observation saw no return of trouble in the 50 per cent. that were apparently completely cured.

Sternberg and Jelkin (51) undertook the treatment of 278 cases, of which 200 were probably gonorrheal. Among these women were 163 suffering from infection of the appendages and periuterine structures. They obtained satisfactory subjective and objective results in 142 of the 163 cases. The treatment lasted from 3 to 18 weeks, on an average of 10 weeks, and required from 5 to 36 injections.

Terebinskaja and Popowa (52) question Sternberg and Jelkin's results. They treated 13 cases of positive gonorrheal tubal infections with the same vaccine and saw no favorable results attributable to it.

Neu (47) tried the effect of vaccines on 26 cases of positive and probable gonorrheal infections of the tubes among the ward cases at the Heidelberg Frauenklinik, and was not able to observe any results that he could credit to the beneficial effects of the vaccine treatment, with the possible exception of one case.

Heymann and Moos (43) obtained no benefit from vaccines in urethritis and endometritis. In 44 recent adnexal swellings they obtained excellent results in 5 instances, improvement worth mentioning in 12 cases, slight but recognizable improvement in 18 cases, while there were 9 that remained unimproved. In 9 old tubal swellings 7 were not benefited, while two were slightly improved. They conclude that the gonococcus vaccine has not proved to be an advance in the treatment of gonorrheal infections of the uterine appendages.

Hauser (41) carefully analyzes the results of treatment in 18 cases of tubal infection, which were probably gonorrheal in origin, and relates that he obtained 5 complete objective and subjective cures and 6 satisfactory cures, in that the patients were relieved of all symptoms, though retaining altered tubes. He believes that vaccine treatment promises from 10 to 20 per cent. better results than does any other non-operative treatment.

Klaus (46) was not able to secure as good results in adnexal disease as in epididymitis and joint infections.

**VulvoVaginitis**

Fitzgibbon (59) treated 6 cases of gonorrheal vaginitis by the use of vaccines. Of these 3 were children, 2 were adults with old infections,
and one was an adult with a recent infection. Four of his cases exhibited a steady improvement until they were cured. Two improved and then relapsed. One of the two, however, eventually recovered. All cases received local treatment in addition to the vaccines.

Hamilton (60) treated 84 cases of vulvovaginitis in children and obtained a complete disappearance of the secretion in 76 instances. The treatment averaged 1.7 months instead of 10 months, as required for other methods of treatment.

Butler and Long (57) report that they were able to cure 11 cases out of 18 treated with vaccines, and the treatment averaged only 14 days.

Churchill and Soper (58) report equally beneficial results in a series of 41 cases.

Boas and Wulf (56) treated 9 cases of vulvovaginitis without clinical benefit, though the opsonic index was increased.

Barnett (55), in 15 cases of vulvovaginitis treated by vaccines, was unable to influence the vaginal secretion, though he secured cures of the joint troubles in a few cases where this complication was present.

The pediatrists apparently have had more success in the treatment of their cases of vulvovaginitis than the gynecologists. While the former have noticed favorable results, the latter almost uniformly report failures in their attempts to influence any of the mucous membrane infections, whether vulvovaginitis in children, or cervical, uterine, or urethral infections in adults.

The most favorable cases for treatment by vaccines are recent tubal infections after the subsidence of fever. When once there is extensive connective tissue alteration with the production of scar tissue no treatment can cause its absorption. Some of the failures are ascribable to the presence of a secondary infection which is not influenced by the gonococcus vaccine. Vaccine does not seem to lessen the chances of tubal involvement when given prophylactically in the beginning of a gonorrhea. When drainage of a pelvic abscess is indicated it should not be deferred in order that vaccines may be tried.

Practically all observers are united in the advice not to give the vaccine in the presence of fever or during the menstrual period.

Other rules for treatment by gonococcal vaccines are the same in pelvic infections as in other gonorrheal affections.

**Lactic Acid Bacilli in Vaginitis**

Many investigators believe that the vagina is in part protected from the invasion of foreign bacteria by the activity of certain Gram-positive bacilli described by Doederlein, which are found in the normal vagina. Sporadic attempts have been made to utilize this organism therapeutically
in infections of the vagina, but the cultivation of this organism is extremely difficult, and no systematic study of this subject has been made. Brindeau (61) has, however, used for this purpose cultures of other bacilli which produce lactic acid. He believes that cultures of the Bulgarian lactic acid bacillus are useful, not only in the vagina, but that they hasten the cleansing of other wounds. He mixes the contents of a culture tube with a solution of milk sugar and pours this into the vagina or over the wound that is to be treated. An overdose, he says, is impossible, since this organism is not pathogenic, and the stronger the culture the more rapid the action. Since the principal rôle of Doederlein's bacillus appears to be the production of lactic acid, which renders the vaginal secretion inimical to the growth of most other bacteria, Brindeau's advice seems to be biologically well grounded when applied to vaginal infections, and worthy of trial, especially since the therapy appears to have no possible bad effects.

TREATMENT OF FEMALE GENITAL TUBERCULOSIS BY THE USE OF TUBERCULIN

The treatment of female genital tuberculosis by tuberculin has not found the warm support that has been accorded the use of tuberculin in some other forms of tuberculosis. Those who have had experience in observing these cases of tuberculosis of the tubes and peritoneum almost without exception support the operative treatment as offering more hope of cure. Franque (62) expresses the opinion of most abdominal surgeons when he says that this variety of tuberculosis is best treated by operation. When there is coexistent lung or other involvement, which in itself is not capable of repair, the pelvic disease is, of course, not suited to operation. But when the genital involvement occupies the most prominent part of the clinical picture an operation for the removal of the local disease should be considered. Neu (65), in a review of the 82 cases of genital and peritoneal tuberculosis treated at the Heidelberg Frauenklinik from 1902 to 1910, found that, of the 55 cases that were operated upon, 75 per cent. were still alive, while of the 21 milder cases that were treated conservatively only 52 per cent. survived. In cases that are considered too advanced for operation tuberculin may be cautiously given under the direction of a physician experienced in its use.

REFERENCES

REFERENCES


Hemorrhagic Diseases of the Newborn


Serum in Uterine Hemorrhage


Induction of Labor by the Injection of Serum


Serum in the Treatment of the Intoxications of Pregnancy

17. Rübsamen. Ibid., 1911, No. 21.

Ringer's Solution in Intoxications of Pregnancy

SERA AND VACCINES IN OBSTETRICS

ANTISTREPTOCOCCUS SERUM AND STREPTOCOCCUS VACCINES


SENSITIZED VACCINES


VACCINES IN Puerperal SEPSIS

34. Western. Lancet, Feb. 10, 1911.

VACCINE TREATMENT OF Gonorrhea in Women

REFERENCES

55. Barnett.
56. Boas and Wulf. Hospitalstidende, July 6, 1910, liii, No. 27; cited by Höffel.

LACTIC ACID BACILLUS IN THE TREATMENT OF VAGINITIS


TUBERCULOSIS OF THE FEMALE GENITALIA AND ITS TREATMENT


THE TRANSFUSION OF BLOOD

INDEX

ABDERHALDEN, ferment of, 15; on immune ferment reactions, 142; test of, for carcinoma, 145; theories of, on relations of invading organism to host in infectious diseases, 16.
ABDOMINAL FISTULA, antistreptococcus serum for post-operative treatment of, 827.
ABRAHAM AND HERMAN, experiments of, in treatment of leprosy with serum, 387.
ABSCESS, alveolar, metastatic processes and, 635, 636; alveolar, specific treatment of, 637; Bartholinitian, metastatic lesions following, 578; perirethral, metastatic lesions following, 578; subcutaneous, in sporotrichosis, 421.
ABSCESES, in actinomycosis, 429; in gonococcal infections, 589; in gonococcal septicemia, 587.
ACHARD AND BENSAUDE, paratyphoeus A and B first described by, 211.
ACNE, micro-organisms in, 624.
ACNE, vaccine treatment of, 628; in cases of comedones, 630; in cases of deep seated nodules and abscesses, 629; in cases of superficial pustules, 629; reaction following inoculations of, 628; value of, 628.
ACNE BACILLUS, cultural characteristics of, 626; cultures of, 626; description of, 625; experimental researches for, 624.
ACNE VACCINE, dosage of inoculations of, 627; estimation of number of bacteria in, 627; preparation of, 626; reaction following inoculations of, 627; standardization of, 627.
ACNE VULGARIS, vaccine treatment in, stock vaccine in, 623.
ACTINOMYCOS, 426, 427; gross appearance of, 426, 429; growth of, 430; lesions due to, 429, 430; microscopical appearance of, 428; morphology of, 427; occurrence of, in nature, 430.
ACTINOMYCOSIS, 426-432; diagnosis of, 431; lesions of, 429, 430; organism of, 426, 427; pathology of, 431; resemblance of, to Madura foot, 432; treatment of, 431, 432; tuberculosis and, 427.
ACTINOMYCOTIN, 431.
ACTIVE IMMUNIZATION. See Vaccine therapy.
ACUTE ARTICULAR RHEUMATISM, gonococcal arthritis resembling, 591.
ACUTE SECONDARY ANEMIA, causes of, 799.
ACUTE ULCEROUS ONGIVITIS, 636; treatment of, 637.
ACUTE YELLOW ATROPHY, fibrinogen insufficiency in, 706.
ADENITIS, TUBERCULOSIS, tuberculin treatment in, 322.
ADENOID, REMOVAL OF, to prevent focal infection, 172.
ADRENAL GLANDS, anaphylactic reaction in, 80.
ADRENALIN for prevention of anaphylactic reaction, 100.
AEROSIS in rabies, 714.
AGE, in gonococcal infections, 598; in prognosis of cerebrospinal meningitis, 559.
AGGLUTINATION, following meningococcus vaccination, 570, 571; in glanders, 657; in sporotrichosis, 423.
AGGLUTINATION OF BACTERIA, by dilute mineral acids, 3; by immune serum, 3.
AGGLUTINATION REACTION IN DIAGNOSIS, 119; specificity of, 120.
AGGLUTINATION REACTION IN DYSENTERY, 124; in Malta fever, 124, 661; in paratyphoid infections, 124; in plague, 125.
AGGLUTINATION REACTION IN TYPHOID FEVER, 120; comparison between gross
and microscopic tests in, 123; diagnostic value of, 123; macroscopic reaction in, 121; microscopic reaction in, 120; typhoid culture in, 120.

Agglutination reaction of gonococci, 603.

Agglutinins, in glands, 657; in Malta fever, 660; in sporotrichosis, 423.


Albuminuria in gonococcal infection, 587.

Alcohol, autolysis of the gonococcus delayed by, 588; pneumonia and, 470; use of, in serum sickness, 558.

Alcohol and carboxic acid injections, in Graves’ disease, 793.

Allergic reactions following use of gonococcal vaccines or gonococcal protein, 600; diagnostic value of, 600.

Allergy, cutaneous, in sporotrichosis, 424; in syphilis, 398, 399.

Alveolar abscess, metastatic processes and, 635, 636; specific treatment of, 637.

Amylopia following use of ethylhydrocuprein for pneumococcus infection, 498.

American losses from typhoid in Spanish-American War compared with losses from other causes, 188–9.

Anacidity, gastric, in pernicious anemia, 808.

Anaphylactic reaction, central or peripheral causation of, 88; experimental analysis of, 51; general symptoms of, in dog, 49; general symptoms of, in guinea-pig, 46; general symptoms of, in man, 50; general symptoms of, in other animals, 50; general symptoms of, in rabbit, 47; phenomena resembling those of, 105; prevention of, in lower animals, 99; prevention of, in man, 101; prevention of, in man, Besredka’s methods for, 102; precautions in prevention of, 101; substances used in prevention of, in lower animals, 100.

Anaphylactic reaction in blood, 82; changes in blood picture in, 84; coagulability of blood and, 82.

Anaphylactic reaction in cardiac system, 60; anatomical changes in, 60; cardiac hemorrhages in, 60; cardiac rate in, 69; functional changes in, 61; functional changes in dog in, 65; functional changes in guinea-pig in, 61; functional changes in rabbit in, 62; muscular changes of heart in, 61.

Anaphylactic reaction in extracardiac circulatory system, 70; blood pressure disturbances in, causes of, 73; blood pressure disturbances in, varieties of, 72, 73; blood pressure in cat in, 72; blood pressure in dog in, 70; blood pressure in guinea-pig in, 72; blood pressure in rabbit in, 71; other changes in, 74.

Anaphylactic reaction in gastro-intestinal system, 78; in dog, 78; in guinea-pig, 79; in rabbit, 79.

Anaphylactic reaction in glandular system, 80.

Anaphylactic reaction in lymph, 85.

Anaphylactic reaction in man, 90; after intraepithelial injections of serum, 94; food idiosyncrasies in, 95; hay fever an example of, 95; manifestations on reinjections in, 91; serum disease a manifestation of, 90; symptoms of, 93.

Anaphylactic reaction in muscle system, 75; in smooth muscle of viscera, 75; in striated muscle, 77.

Anaphylactic reaction in nervous system, 85.

Anaphylactic reaction in respiratory organs, in dog, 59; in guinea-pig, 51; in guinea-pig, in subacute anaphylaxis, 58; in man, 59; in rabbits, 59.

Anaphylactic reaction in temperature, 86.

Anaphylactoid phenomena, 105.

“Anaphylatoxin” of Friedberger, 16, 17.

Anaphylatoxins, 474.

Anaphylaxis, 804, 805, 807; complicating cerebrospinal meningitis, 558–562; criteria of, 103; definition of, 38; focal infection and, 171; functional analysis of, 35; history of, 36; introduction to, 35; parenteral digestion theory of, 107; production of, by injection of washed typhoid bacilli, 201; theories of, 106; Vaughan’s theory of, 107.

Anaphylaxis, experimental, 38; active, 39; dosage in sensitization in, 40; incubation in, 43; influence of various
INDEX

manipulations on sensitizing substances in, sensitization in, 43; intoxication in, 44; method of, intoxication in, 45; methods of, sensitization in, 41; precautions against, 568, 569; sensitization in, 39; sensitizing and intoxicating fraction of foreign protein in intoxication in, 46; sensitizing substances used in, 39; specificity of reaction in sensitization in, 42; symptoms of intoxication in, 45. See also Serum sickness.

ANAPHYLAXIS, LOCAL, 87.

ANAPHYLAXIS, PASSIVE, 96; animals used in, 96; duration of, 97; in excised organ, 97; refracting period between injections in, 96; sensitization transferable in, 96; symptoms in, 97.

ANASARCA, due to gonococcal infection, 587.

ANDERS', statistics of, on serum therapy in pneumonia, 476.

ANDERSON on anaphylaxis, 98.

ANEMIA, acute secondary, causes of, 799; aplastic, 809; chronic secondary, causes of, 799; chronic secondary, following hemorrhage, serum and blood treatment of, 811; classification of, 798, 799; excess of antithrombin in, 797; general methods of treatment of, with normal sera and blood, 802–808; in gonococcal infection, 589; in Graves' disease, 791; in purpura hemorrhagica, 814; pernicious, defibrinated blood in treatment of, 808, 810; pernicious, diagnosis of, 808; pernicious, transfusion of blood in, 809, 810; primary idiopathic, serum and blood treatment of, 808–810; splenic, serum and blood treatment in, 810.

ANESTHESIA for lumbar puncture, 524.

Anesthizin ointment, use of, in serum sickness, 558.

ANGINE, on allergic reactions in gonococcal infections, 601.

ANTHEAXIS, serum therapy in, 655.

ANTIANAPHYLAXIS, 93; duration of, 93; procedure of Besredka in, 98.

Antiantherax serum, 655.

Antibacterial serum, 473.

Antibodies, Ehrlich's grouping of, 7; formation of, in host, upon invasion of infecting organisms, 6; specificity of, 753. See also Iso-antibodies and Auto-antibodies.

Antidiptheric serum. See Diptheria antitoxin.

Antidysenteric sera, production of, 259.

ANTIGEN REACTIONS, physico-chemical processes in, 4.

ANTIGENS, chemical nature of, 4; definition of, 4; modification of, by physico-chemical means, 5.

Antigonoecocci serum, 618, 619.

Antilens serum, 761; Pick's experiments with, 759, 760.

Antimeningitis serum, administration of, 560; blood pressure and, 528, 529; cases showing use of, 530, 531, 534, 535, 537, 538, 539, 541, 544, 545, 551, 552, 554, 555–557; concentrated, 532; dosage of, 523, 524, 528, 529, 533, 534; favorable results following use of, 533; injection of, by gravity method, 527, 528; injection of, by lumbar puncture, 522–526; injection of, by syringe method, 527; injection of, following dry puncture, 535; injection of, in cases with thick, plastic exudate, 534, 535; intraspinal injection of, contraindicated in posterior basic meningitis, 535, 542; local application of, in arthritis complicating meningitis, 553; local application of, in eye complications of cerebrospinal meningitis, 550, 551; mechanical effects of, 530; method and technique of administering, 527–529; potency of, 560; preparation of, 521, 522; prophylactic use of, 566, 568, 575; refined or concentrated, 532; serum sickness due to, 554–558; signs of response to, 561; subcutaneous and injection of, 522; symptoms accompanying injection of, 528, 529; use of, for bacteriemia preceding and during course of cerebrospinal meningitis, 533–540; use of, in chronic meningitis, 540–541; use of, in heart complications of cerebrospinal meningitis, 553, 554; use of preservatives with, 532, 533.

Antimeristem, in treatment of cancer, 783.

Antipneumococcus serum, 473–487; administration of, 484; dosage of, 479–481; effects of, 484, 485: Neufeld and,
INDEX

Antitumor serum, cytolytic effects of, on tumor cells, 758, 759.
Antityphoid vaccination, 178; history of, 178–182.
Antityphoid vaccination in British Army, in Boer War, 180; conclusions from unfavorable results in, 181.
Antityphoid vaccination in German Army, 182.
Antityphoid vaccination in India, 193.
Antityphoid vaccination in United States Army, first use of, 182; history of, 188–190; administration of vaccine in: method of injection of, 184; time of injection, 183; preparation of vaccine in: growing culture in, 182; killing of culture in, 182; standardization in, 182; tests for purity of, 183; tests for sterility of, 183; reaction following administration of vaccine in: in blood serum, 185–186; general, 184–185; local, 184; results of, 188–192.
Antityphoid vaccine, dosage of, for children, 195; duration of immunity from, 196; in case of those already infected, 196; indications for use of, in service and civil life, 195; preparation of, 225; revaccination with, 196; sensitized killed, 187; sensitized killed, of F. P. Gay, preparation of, 187; sensitized living, 186; use of, in civil life in United States, 190; use of, in epidemics as prophylactic measure, 196; use of, in France, 193.
Antivenin, cobra, administration of, 747; preparation of, 747.
Antivenin, rattlesnake, 747.
Aplastic anemia, 809.
Appendicitis, surgical treatment of, to prevent focal infection, 173.
Arnonson on antistreptococcus serum, 650.
Arrhenius on mass action in passive immunity, 8.
Arsenic, in treatment of scarlet fever, 647.
Arthigia, use of, in gonococcal arthritis, 614.
Arthritic purpura, serum treatment of, 814.
Arthritis, alveolar abscess and, 635, 636; chronic, polyvalent streptococcus horse serum in, 176; complement-fixation test.
INDEX

in, 140; complicating cerebrospinal meningitis, 552, 553; deforming, vaccine therapy for, 175; hemolytic streptococci cause of, 507; pyorrhea and, 635.

Arthritis, gonococcal, 578–581; septicemia following, 585; serum treatment of, 618, 619; vaccine treatment of, 613–615.

Arthritis deformans, gonococcal arthritis and, 550; vaccine therapy for, 175.

Arthus, experiments of, on anaphylaxis, 37; on dosage of sensitization in experimental anaphylaxis, 41.

Articular fluids, examination of, for gonococcus, 595; in gonococcal arthritis, 581.

Ascites-blood-agar plates, preparation of, 594.

Ascites fluid, use of, in culture media for gonococcus, 592.

Aseptic meningitis, 513.

 Asiatic cholera, immunizing properties of cholera sera in, 280; indications for methods of treatment in, other than serum, 292; intravenous injections of saline solution in treatment of, 293; Kraus serum for treatment of, 284; nature of toxin in, 280; Rogers' method of treatment of, without serum, 291; serum treatment of, 280; symptoms of, 292, 293; treatment of, by enemata of serum, 293; treatment of, in Russia, with sera, 289; treatment of, with cholera immune sera, 288; vaccination for prophylaxis in, 280; vaccine treatment of, 280.

Aspirin in treatment of cerebrospinal meningitis, 548.

Atropin, use of, following injection of antitoxin, and meningitis serum, 529; for prevention of anaphylactic reaction, 100; in serum sickness, 558.

Atenuation of rabies virus, by dialysis, 729, 730; by glycerin, 729; by gradual drying, 726–728; by heat, 728, 729; by rapid drying, 728, 729.

Auer and Lewis on heart block in anaphylactic reaction, 62.

Auer and Robinson on functional cardiac disturbances in anaphylactic rabbit, 63, 64.

Austin and Frothingham on serum agglutination test for typhoid fever, 207.

Austrian, on ophthalmic diagnostic reaction in typhoid, 210; on technique of typhoid-opthalmal reaction, 158.

Auto-antibodies, effects of, in vivo, 767; in experimental cancer in vitro 767, 768.

Autogenous vaccines, in fusiform bacillus infections, 637; in gonococcal arthritis, 613; in treatment of typhoid carrier, 223.

Autolysins, action of, in experimental cancer in vivo, 768, 769.

Autolysis, of the gonococcus, 597.


Autovaccination, for cancer, 781, 782.

Autumn catarrh, pollen of Dicotyledones cause of, 673, 675.

Avian tubercle bacilli, inoculations of, used in preparation of serum for treatment in leprosy, 387.

Axenow on use of antistreptococcus serum in scarlet fever, 646.

Bab, report of acne cases treated with vaccines by, 630.

Babes, experiments of, in treatment of leprosy with serum of animals inoculated with avian tubercle bacilli, 387; on antirabic serum, 734.

Babes–Kedrowsky type of acid-fast or acid-resistant diphtheroid bacillus in leprosy cases, 383.

Babes’ serum-vaccine treatment for rabies, 732.

Bacillary dysentery, 249.

Bacilli, fusiform, infection of the mouth by, 635, 636; specific treatment of, 637.

Bacilli of dysentery. See Dysentery bacilli.

Bacillus coli communis, association of, in septic processes, 232; bacterial therapy in lesions produced by, 232; cultural characteristics of, 233; cystitis caused by, 220; differentiation of, from gonococcus, 595, 597; discovery of, 232; in pyelitis or pyelonephritis, differentiated from other organisms, 242;
invasion of the genito-urinary tract by, diseases caused through, 238; migration of, into blood stream, conditions necessary for, 233; occurrence of, 232; organisms associated with, 235; pyelitis caused by, 239; spread of infection of, to tissues other than the intestinal tract, 237; tolerance to, of tissues outside intestinal tract, 235; urethritis caused by, 238; wounds infected by, vaccine therapy of, 246.

BaciLLus enteritidIs, association of, with colon bacillus, 235; cultural characteristics of, 233.

BaciLLus leprae, discovery of, by Hansen, 383; uncertainty of successful cultivation of, 385.

BaciLLus proteus, association of, with colon bacillus in lesions, 235.

BaciLLus typhosus. See Typhoid bacilli.

BaciLLus y of dysentery, 248.

bacIlIester on tubercle bacilli in the blood, 312.

Bacteria, in the normal mouth, 635; selective action of, in body tissues, 201; tissue disintegration caused by, 202.

Bacterial filtrates, non-specific, 34.

Bacterial infection, Abderhalden's theories of cause of: by breaking up of body cells by ferment of invading organisms and utilizing them for bacterial proteins, 16, by proteolysis or breaking up of tissues of body indirectly brought about by ferment of invading organism, 16, by proteolysis products of foreign bacterial protein in body, 16; Vaughan's theory of cause of: by proteolysis of bacterial protein in body, 16.

Bacterial meningitis, 513.

Bacterial protein, freed in tissue cells, separation in vitro of, by Vaughan's method, 201; immunity produced by final digestion of, by body ferment, 202; products of digestion of, in body, cause of intoxication, 16; symptoms produced by injections of, 201.

Bacterial proteolysis of typhoid bacillus in tissues, cause of typhoid manifestations, 200.

Bacterial suppurrative meningitis, bacte-terial examination of cerebrospinal fluid in, 518.

Bacterial therapy in typhoid fever, 199.


Bacteriological diagnosis of typhoid fever, 202.

Baetz on use of vaccines in treatment of gonococcal arthritis, 614.

Baetz and Bates on typhoid agglutination tests in diseases other than typhoid, 210.

Baginsky on use of antistreptococcus serum in scarlet fever, 646.

Bail's experiments of bacterial inoculations in immunity, 24.

BalAnitis, metastatic lesions following, 578.

BanDeIler on results covering tubercle bacilli in sputum following tuberculin treatment, 320, 321.

Barfurth and Schottmüller, on use of vaccines in gonococcal pelvic infections, 617.

Barium chloride for prevention of anaphylactic reaction, 100.

Barnett on vaccine treatment of anaphylactic reaction, 835.

Bartholinian abscess, metastatic lesions following, 578.

Bates, on blood cultures and agglutination tests in mild atypical typhoid fever, 204.

Bayon, treatment of leprosy by, with extract from Kedrowsky's culture, 392.

Beard, G. M., on hay fever, 670.

Beere, on specificity of nucleo-proteins, 756, 757.

Beere and Rogers' serum for Graves' disease, 794.

Beef agar for culture of gonococcus, 592.

Behring, active immunity against diphtheria with toxin-antitoxin mixtures applied to man by, 442; experiments of, on anaphylaxis, 36; method of standardization of diphtheria antitoxin of, 437; modification of, of Römer's intracutaneous method of estimating amount of antitoxin in blood, 443; Tuberculose of, 324; Tulasealatin of, 324.

Behring and Ehrlich, method of, of
INDEX

standardisation of diphtheria antitoxin, 438.
Behring and Wernicke, discovery of antitoxic diphtheritic serum by, 434.
Beranecck’s Tuberculin, preparation of, 325.
Bertrand on use of unaltered vaccines in cancer, 776.
Besredka, investigations of, in sensitised vaccines, 29; method of, for preparing sensitised gonococcal vaccines, 609; methods of, for prevention of anaphylactic reaction, 102; procedure of, in antianaphylaxis, 98.
Biedl and Kraus, on dosage of sensitisation in experimental anaphylaxis, 41; on anaphylactoid phenomena, 105.
Berry and Pettit on specificity of nucleoproteins, 756.
Bills, obtaining cultures of typhoid bacillus from, 204.
Biological treatment of cancer, bibliography on, 784–788; clinical data on, 773–782; criteria of therapeutic effectiveness, 770–772; experimental data on, 751–769; introduction to, 750, 871; relationship between clinical and experimental data, 769; summary, 782–784.
Birds, immunity of, to rabies, 706.
Blackley, Charles, experimentation of, on pollen as etiological factor of hay fever, 670.
Bladder, care of, in cerebrospinal meningitis, 547; inflammation of, caused by colon bacillus, 238.
Blajitz and Nicolle, method of, in preparing gonococcal vaccines, 608, 609.
Blastomyces, examination and isolation of, 425; pathogenic properties of, 425.
Blastomyces, examination and isolation of organism in, 425; morphology of, 425; pathogenesis of, 425; treatment of, 426.
Bleeding, in prophylactic treatment of rabies, 718.
Blindness following cerebrospinal meningitis, 551.
Blood, anaphylactic reaction in, 82; defibrinated, in therapy of hemorrhagic disease of the newborn, 823; defibrinated, in treatment of hemorrhagic diseases, 821; see also Defibrinated blood; examination of, for gonococcus, 594, 596; in gonococcal infections, 588, 589; mechanism of coagulation of, 795–796; of lepers, used in inoculation of horses in preparation of serum for treatment in leprosy, 387; transfusion of, from normal woman in intoxications of pregnancy, 825; transfusion of, in hemorrhagic disease of the newborn, 823; see also Transfusion of blood; whole, in treatment of hemorrhagic diseases, 820; whole, intravenous use of, 807, 808.
Blood agar for culture of gonococcus, 594.
Blood clot formation, physiology of, 630.
Blood cultures in bacteriological diagnosis of typhoid fever, 203.
Blood picture, changes in, in anaphylactic reaction, 84.
Blood platelets, decrease of, in pernicious anemia, 808; decrease of, in purpura hemorrhagica, 814; hemorrhage and purpura and, 797.
Blood pressure, dosage of antimeningitis serum and, 523, 524; injection of antimeningitis serum and, 528, 529; withdrawal of cerebrospinal fluid and, 727.
Blood therapy, 798.
Blood vessels, anaphylactic reaction in, 70.
Blumenthal, experiments of, with cancer vaccination, 765; method of, for preparing autolyzed vaccine for cancer, 779, 780.
Blumer and Thayer, gonococcus isolated from blood by, 579.
Boardman, quantitative cutaneous tuberculin tests of, for administration of tuberculin, 347.
Boer War, typhoid cases in British Army in, statistics of, 180; statistics of inoculated and unprotected in, 180; statistics of typhoid losses in, compared with losses from other causes, 188–189.
Bollinger, actinomycoses first isolated by, 426.
Bolton, on gastrotoxic serum, 762.
Bones and joints, tuberculosis of, tuberculin treatment in, 322.
BONY ANKYLOSIS, following gonococcal arthritis of the spine, 580.
BORDET AND GENGOU, discovery of whooping-cough bacillus by, 296; on complement-fixation reaction, 126.
Bosc, use of serum therapy by, in cancer, 773.
BOSTOCK, JOHN, first to report hay fever, case, 665.
"BOSTOCK'S CATARRH," 665, 674.
BOWELS, care of, in cerebrospinal meningitis, 547.
BRAU AND DENIR, experimentation of, with cholera immune sera, 288; production of cholera toxin by, from cholera vibrio, 282.
BRAUN AND HUBLER, test by, of cerebrospinal fluid, 517.
BRINDRAU on therapeutic use of lactic acid bacilli, 834.
BRITISH ARMY LOSSES FROM TYPHOID IN BOSNIAN WAR compared with losses from other causes, 158–159.
BRITISH COMMISSION on results of serum treatment of plague in India, 275.
BRITONNEAU, first to describe diphtheria, 434.
BROMIDS, in treatment of Graves' disease, 792; in treatment of cerebrospinal meningitis, 548.
BROUGHTON-ALCOCK, work of, on sensitized vaccines in typhoid immunization in man, 30.
BROWN, on results covering life duration following tuberculin treatment at Saranac, 319; on results covering tubercle bacilli in sputum following tuberculin treatment at Saranac, 320.
BRUCK, on allergic reactions in gonococcal infections, 601; on vaccine treatment of gonococcal arthritis, 613.
BRUCK'S "ARTHITON," 610.
BUCHEMER AND HAHN, tuberculin of, 326.
Bulgarian buttermilk in treatment of Graves' disease, 792.
Bulgarian sour milk diet in treatment of typhoid carriers, 222.
BUNTING AND YATES, experiments by, in Hodgkin's disease, 662.
BURNETT, results of serum treatment of plague in Queensland, 274.

BUTLER AND LONG on vaccine treatment of vulvovaginitis, 833.
CALCULUS, in the blood, 796.
CALCULI, feeding in icterus, 796.
CALMETTE, researches of, in snake venom antiserum, 747.
CALMETTE'S OPTHALMO-TUBERCULIN REACTION, 158.
CALMETTE'S TUBERCULIN, 324.
Calomel, in treatment of ulcerous gingivitis, 637.
CAMPANA, isolation by, from leprosy cases, of anaerobic acid-fast organisms, 353.
Camphor, use of, in pneumonia, 501.
CANCER, changes in size of, following treatment, 770–772; circulatory changes in, following treatment, 770; criteria of therapeutic effectiveness in 770–772.
CANCER OF THE UTERUS, antogenous vaccine and antistreptococcus serum in post-operative treatment of, 827.
CANCER ANTISERA, intravital action of, in animals, 761–762; specificity of, 761, 762.
Cancroin, in treatment of cancer, 783.
Carbolic acid, attenuation of rabies virus by, 729.
Carbolic acid and alcohol injections in Graves' disease, 793.
CARBUNCULOSIS, vaccine therapy for, 632.
CARCINOMA, Abderhalden test for, 145; blastomycosis and, 425, see also Cancer; mioestamin reaction in, 146, see also Mioestamin reaction in carcinoma.
CARDIAC INHIBITION, following injection of antimeningitis serum, 530.
CARDIAC SYSTEM, anaphylactic reaction in, 60.
CARIOUS TEETH, correction of, to prevent focal infection, 172.
CARRASQUILLA, experiments of, in treatment of leprosy by serum, 387.
CARRIÈRE AND TOMARKIN, production by, of serum for Asiatic cholera, 285.
INDEX

CARRIERS, MENINGOCOCCUS, 563, 564; typhoid, see Typhoid carriers.
CASTELLANI AND WOOLLEY on use of lepromata in treatment of leprosy, 389.
CATARRH, SUMMER, etiology of, 673, 674. "CATARRHUS MESTIVUS," 667; etiology of, 673, 674.
CATS, rabies in, 705.
CATTLE, rabies in, 705.
CAUTERIZATION OF WOUNDS in prophylactic treatment of rabies, 718.
CAZENAVE, of Bordeaux, on hay fever, 668.
CELL COUNT of blood, in cerebrospinal meningitis, 518–519; of cerebrospinal fluid, in syphilis of central nervous system, 404, 405.
CENTRAL NERVOUS SYSTEM, anaphylactic reaction caused by, 88; syphilis of, see Syphilis of central nervous system.
CEREBRAL EMBOLISM in gonococcal endocarditis, 588.
CEREBROSPINAL FLUID, 400; bacteriological examination of, 517, 518; cell count of, 404, 405, 416; changes in, after administration of antimeningitis serum, 561, 562; chemical examination of, 516, 517; clearing of, after treatment, 533; color of, 516; cytological examination of, 518–519; effect of serum treatment on, 520, 521, 533; fibrin content of, 516; guide to treatment in syphilis, 400; in chronic meningitis, 520; in fully developed epidemic meningitis, 520; in premeningitic stage, 519; pressure of, 515, 516; protein content of, 516; technique of examining, 515–521; withdrawal of, in cerebrospinal meningitis, 527.
CEREBROSPINAL FLUID IN SYphilIS, 399, 404–407; globulin content of, 405; pleocytosis of, 404, 405; pressure of, 416; Wassermann reaction in, 405–407.
CEREBROSPINAL MENINGITIS, aseptic, 513; bacterial, 513; bacterial supplicative, 518; cases of, 530, 531, 534, 535, 537, 540, 541, 543, 546, 547, 551, 552, 554, 555–557; cerebrospinal fluid in, 520; classification of, 513, 514; complications of, 548–558; diagnosis of, 514, 515; due to gonococcus, 588; epidemic prophylaxis of, 563–576; examination of cerebrospinal fluid in, 515–521; fulminating, 536; general treatment of, 547, 548; method and technique of administering antimeningitis serum in, 527–529; mortality in, 560; mortality in, with and without serum treatment, 562, 563; non-bacterial, 513; posterior basic, 535; treatment of, 542–545, 561; premeningitic symptoms of, 536; primary, 514; prognosis of, 559–563; references on, 576; relapse in, 558; secondary, 514; secondary syphilitic, cell count of cerebrospinal fluid in, 404, 405; serum treatment of, 521, 540; serum treatment of, and prognosis, 559, 560; serum treatment of general bacteriemia preceding and during course of, 535–540; staphylococcic, 514; streptococcic, 514; subacute, treatment of, 540–545; treatment of complications of, 548–558; treatment of convalescents in, 548; treatment of hydrocephalus in, 539, 540; treatment of relapse in, 558; treatment of, by sinus drainage, 544; with dry canal, treatment of, 535; with thick plastic exudate, treatment of, 534, 535.
CEREBROSPINAL MENINGITIS, CHRONIC, cerebrospinal fluid in, 520; classification of, 540; mild form of, 541; severe form of, 540; treatment of, 540, 545; vaccine treatment of, 540.
CEREBROSPINAL SYphilIS. See Syphilis of central nervous system.
CERNOVODEANU AND HENNI ON absorption of tetanus toxin, 456.
CHANCRO, 397.
CHANTMESSE, experiments of, on ophthalmic diagnostic reaction in typhoid, 209; on immune serum therapy in typhoid fever, 226; typhoid-ophthalmic reaction of, 158.
CHEMOTHERAPY, in pneumococcus infections, 496–500.
CHLORAL, in treatment of cerebrospinal meningitis, 548.
CHLORAL HYDRATE for prevention of anaphylactic reaction, 100.
CHLOROSIS, treatment of, 808.
CHOKSY, on early and free use of plague immune serum, 272; results of investi-
INDEX

Gations of, on serum treatment of plague, 272.

Chologogue cathartics in treatment of acute typhoid carrier, 222.

Cholecystectomy in treatment of typhoid carriers, 222.

CHOLECYSTITIS AND CHOLANGITIS, surgical treatment of, to prevent local disease, 172.

Cholera immune sera, effect of, in man, 288; immunizing properties of, 288; in treatment of Asiatic cholera, 288.

Cholera serum of Braun and Denier, preparation of 288; results with, 288, 289.

Choroiditis, suppurative, complicating cerebrospinal meningitis, 550.

CHRONIC MENINGITIS. See Cerebrospinal meningitis, chronic.

CHRONIC SECONDARY ANEMIA; causes of, 799; following hemorrhage, serum and blood treatment of, 811.

Choriois, fibrinogen insufficiency in, 796.

Cisterna, for hydrocephalic conditions, 544.

Cleag, isolation by, from leprosy cases, of acid-fast organisms which produce yellow or orange-colored colonies, 383.

Cleag bacillus of leprosy, treatment of leprosy with vaccines prepared from, 390.

Clot-cultures of typhoid bacillus in diagnosis of typhoid fever, 205.

Clot formation, physiology of, 820.

Coagulability of blood, anaphylaxis and, 82.

Coagulation of the blood, mechanism of, 795–796.

Cobra antivenin, administrations of, 747; preparation of, 747.

Cobra venom, action of, on blood cells, 744, 745; separation of hemolytic from neurotoxic constituents of, 744; solutions of, in diagnosis of syphilis, 745; solutions of, in diagnosis of tuberculosis, 746.

Coca and Gilman, use of unaltered vaccines by, for cancer, 777.

Coca, Drorance, and Lебredo, report of, on vaccine therapy in cancer, 777, 778.

Cocain, use of, following injection of antituberculin serum, 529.

Codein, use of, following intraspineous injection of serum in cerebrospinal syphilis, 418; in cerebrospinal meningitis, 548; in serum sickness, 558.

Cold, pneunmonococcus in, 469; pneumonia and, 470.

Coleman and Buxton on post-typhoidal elevations of temperature due to reappearance of bacilli in blood stream, 205.

Coley’s toxins, in treatment of cancer, 783, 784; in treatment of Hodgkin’s disease, 663.

Colloidial gold reaction of Lange, 3; diagnostic differentiation by means of, 4; Zeigmondy findings on, 3.

Colon bacillus. See Bacillus coli communis.

Colon-typhoid bacilli, differentiation of, by agglutination reaction, 233; by cultural characteristics, 233.

Complement, anaphylactic reaction in, 84.

Complement-fixation in echinococcus disease and other tenias, 140; clinical value of test in, 141; preparations of antigen in, 140; technique of test in, 140.

Complement-fixation in glanders, 657.

Complement-fixation in gonococcal infections, 137, 600; clinical value of tests in, 139; significance of test in, 139; preparation of antigen in, 138; technique of: titration of amoebceptor, 138; titration of antigen, 138, carrying out of test, 139.

Complement-fixation in malta fever, 660.

Complement-fixation following meningococcus vaccination, 571.

Complement-fixation in sporotrichosis, 423, 424.

Complement-fixation in syphilis, 127.

Complement-fixation in tuberculosis, 141.

Complement-fixation reaction, as index of efficiency of vaccine inoculation in gonococcal infections, 610; in diagnosis, 125; in diagnosis of gonococcal infections, 603–606.

Complement-fixation test of blood serum after meningococcus vaccination, 573; technique of, 573, 574.
INDEX

CONJUNCTIVAL-TUBERCULIN REACTION, 158.
CONJUNCTIVITIS, complicating meningitis, 550.
CONRAD, obtaining of dysentery toxin by, 264.
COPPER SULPHATE, in treatment of blastomycosis, 426.
CORYNEBACTERIUM GRANULOMATIS MALIG., 662.
CHILE, on Graves' disease, 790.
CUMMING, modification of fixed virus by, by dialysis, 729, 730.
CURRIE, CLARK AND HOLLERMANN, experiments of, in treatment of leprosy with serum made by injecting live cultures of acid-fast bacilli in normal saline solution, 388; treatment of leprosy with vaccines by, 390.
CUTANEOUS ALLERGY. See Allergy.
CUTANEOUS DIAGNOSTIC REACTION IN TYPHOID, 210.
CUTANEOUS GONOCOCCAL REACTION, 159.
CUTANEOUS REACTION FOLLOWING USE OF GONOCOCCAL VACCINES, 600-602.
CUTANEOUS TEST FOR SYphilis, 160.
CUTANEOUS TUBERCULIN TEST, 155.
CYSTITIS, DUE TO BACILLUS COLI, 238; vaccine treatment of, 244.
CYTOLYSINS, specificity of, 753.
CYTOLYTIC EFFECTS, of antitumor sera upon tumor cells, 758, 759; of iso- and auto-antibodies, 766, 767.
CYTOLYTIC PROPERTIES OF ANTISERA, 757, 759.
DALE on anaphylactic reaction on smooth muscle of viscera, 78.
DAVIES, treatment of leprosy by, with injections of extract made from Bayon's bacillus, 392.
DEAFNESS complicating cerebrospinal meningitis, 551, 552.
DEEHAN on cutaneous diagnostic reaction in typhoid, 210.
DEAFFBRINATED blood, 805, 806; in treatment of acute anemia following hemorrhage, 811; in treatment of chronic secondary anemia following hemorrhage, 811; in treatment of hemorrhage in typhoid fever, 814; in treatment of hemorrhagic diseases, 821; in treatment of hemorrhagic disease of the newborn, 823; in treatment of pernicious anemia, 809, 810; in treatment of splenic anemia, 810; intravenous administration of, 806; mode of action of, 805, 806; preparation of, 806.
DELETER, use of unaltered vaccines by, in cancer, 776.
DELIRIUM in gonococcal infections, 588.
DEMBSKAJA on vaccine treatment of gonorrheal infection of female genitalia, 832.
DENIER, statistics of cholera cases treated by, with Serums A and B, 288.
DENISON on results with tuberculin treatment, 317.
DENYS on results of tuberculin treatment, 317.
DENYS AND LECLEF, work of, on antistreptococcus serum, 650.
DENYS' BOUILLON FILTRATE, 330; preparation of, 324.
DERMATOLOGY, vaccine therapy in, 623.
DERMATOSES OF PREGNANCY, normal serum in treatment of, 825.
DESENSITIZATION for antimeningitis serum, 557.
DETRA on complement-fixation in syphilis, 127.
DEUTSCHMANN'S serum, 654.
DE WITT, LYDIA M., on antagonism between diphtheria bacilli and staphylococci in throat, 449.
DEYCKE AND MUCHE, tuberculin of, 326.
DEYCKE-PASCHA, acid-fast streptothrixes isolated in leprosy cases by, 383.
DEYCKE-PASCHA AND RESCHAD-BET, experiments of, with the "streptothrix leproideus," 385.
DIAGNOSIS, immunological reactions in, 117.
DIAGNOSTIC REACTIONS IN PATIENT, 151; • conjunctival-tubercul in reaction in, 158; cutaneous gonococcal reaction in, 159; cutaneous tuberculin test in, 155; in gonococcal infections, 159; in syphilis, 100; in tuberculosis, 151; in typhoid fever, 158; intracutaneous gonococcal reaction in, 159; intracutaneous tuberculin reaction in, 157; luetin reaction in, 160; ophtalmo-tubercul in reaction in, 158; pereutaneous tuber-
culin test in, 157; subcutaneous gono-
coccal reaction in, 159; subcutaneous tuberculin test in, 151; typhoid-oph-
thalmo reaction in, 158.

Diagnostic reactions in vitro, 117; ag-
glutination reaction in, 119; agglutina-
tion reaction in, specificity of, 120; complement-fixation reaction, 125; complement-fixation in echinococcus disease and other toxins: chemical value of test in, 141, preparation of antigen in, 140, technique of test in, 140; complement-fixation in gonococcal infections: clinical value of tests in, 139, preparation of antigen in, 138, significance of test, 139, technique of, 138; complement-fixation reaction in syphilis, 127, see also Wassermann reaction; complement-fixation in tuberculosis, 141; complement-fixation with spirochetal antigen in, 127, influence of treatment on, 135, nature of, 133, technique of, 127; epiphanin reaction, 147, preparation of antigen in, 148, technique of, 148; immune-ferment reactions: 141, Abderhalden test for carcinoma in, 145, serodiagnosis of pregnancy in, 142; methods of obtaining serum in: in large quantities, 118, in small quantities, 117; miostagmin reaction, 145, in carcinoma, 146; in tuberculosis, 146; opsonic reaction: 148, clinical value of, 150, significance of, 149, technique of, 149; precipitin reactions, 125; Wassermann test in diseases other than syphilis, 134; Wassermann reaction in syphilis: clinical value of, 134; Widal reaction, 119, 120.

Dialysis, modification of fixed virus by, 729, 730.

Diarrhea, in gonoccocemia, 589.

Diaz reaction of Ehrlich in gonococcal septicemia, 591.

Dioctyledones, structural characteristics and composition of pollen of, 673, 674.

Diet, in gonococcal infections, 607; in Graves' disease, 792.

Diffuse erythema, in gonococcemia, 588.

Diphtheria, antitoxin treatment of, 445, see also Diphtheria antitoxin; discovery of serum therapy of, 434; Schick's test of immunity to, 441.

Diphtheria, eradicating diphteria bacilli from throat in, by staphylococcus inoculations, 449; by staphylococcus sprays, 449; by subcutaneous antitoxin injections, 449; by vaccines of chloroform devitalized diphtheria bacilli, 450; by vaccine of diphtheria bacilli prepared in intense cold and filtration, 450.

Diphtheria, specific prophylaxis against, with antitoxic serum, 440; conditions calling for, 440, 441; disadvantages of, 440; Schick's test to limit use of, 441.

Diphtheria, specific prophylaxis against with toxin-antitoxin serum, 442; heterologous, 443; homologous, 443; immunizing effects of, 443, 444.

Diphtheria antitoxin, 445; dosage of, factors governing, 445; early administration of, 445; effect of, on nephritis in diphtheria, 446; effect of, on postdiphtheritic paralysis, 446; intramuscular injection of, 447; intravenous method of administering, 447; methods of administration of, 448; subcutaneous injection of, 447.

Diphtheria antitoxin, estimating unit of, Behring's method of, 437; Behring and Ehrlich's method of, 438; Ehrlich's method of, 438; Ehrlich and Wassermann's method of, 437, 438; present method of, 438; Roux's method of, 437.

Diphtheria antitoxin, practical production of, 435; animals used in, 435; collection of blood in, 435, 436; concentration of diphtheria antitoxin in, by method of Gibson and Banzhaf, 436; distribution of, 439; Ehrlich's standard unit of, 438; government regulation of manufacture of, 440; keeping qualities of, 439; obtaining antitoxic plasma in, 436; official standard unit of, in Germany, 438; official standard unit of, in the United States, 438; standardization of, 437.

Diphtheria bacillus, action of, 434; history of, 434.

Diphtheroid organism in Hodgkin's disease, 662, 663.
INDEX

DIPLOCCUS PNEUMONIE. See Pneumococcus.

DOCHERZ AND GILLESPIE, studies by, on antipneumococcus serum, 481, 482.

DOES, list of sensitizing substances of, used in experimental anaphylaxis, 39; on frequent increased susceptibility following inoculation of bacteria or their toxins, 15.

DOGS, rabies in, 705.

DONKEY serum, use of, in pneumonia, 476.

DORRANCE, COCA AND LEREDO, on vaccine therapy in cancer, 777, 778.

Douches, nasal, following meningocoeccus vaccination, 575; prophylactic use of, in meningitis epidemics, 566.

DOYEN'S vaccines, in treatment of cancer, 783.

DROP JAW in rabies, in dogs, 714.

DRY PUNCTURE, in cerebrospinal meningitis, 535; in posterior basic meningitis, 542.

DUCAYEY, isolation by, from leprosy cases, of anaerobic acid-fast organisms, 383.

DUMB HYDROPHOBIA, 702.

DUMB BABIES, 704, 713, 714, 715.

DUNBAR, on composition of pollen, 777; on pollen as cause of hay fever, 672.

DUNBAR'S antitoxic immune serum, pollantin, for hay fever, 684.

VON DUNGERN, experiments of, with serum prepared against ciliated epithelium, 757, 758.

DUNGILSON, ROBLEY, on hay fever, 669.

DUODENAL FLUID, finding of typhoid bacillus in, 204.

DUODENAL ULCER, transfusion of blood in, 815.

DUVAL, investigations of, on acid-fast type of bacilli in leprosy cases, 383; on the true bacillus of leprosy, 386.

DYE, experiments of, in treatment of leprosy with venous serum, 389.

DYSENTERY, agglutination reaction in, 124; dosage in serum treatment in, 262; effect of serum treatment on body temperature in, 260; effect of serum treatment on mortality in, 260; results of serum treatment in, 260; results of vaccine treatment in, 263; serum treatment in, 260; serum treatment of, in man, 260; specific action of serum treatment for corresponding strains of bacilli in, 260, 261; vaccine treatment in, 262.

DYSENTERY antitoxin, instability of, 259.

DYSENTERY BACILLI, cause of dysentery in man, 251, 252; history of investigations on, 249; types of, 249.

DYSENTERY BACILLI, differentiation of types of, 252; by absorption method of Castellani, 252; as acid and non-acid varieties, 253; by agglutination tests, 250, 252; by serological tests, 253; by toxicity, 253; in relation to treatment, 253.

DYSENTERY IMMUNE SERUM, 257; antitoxic action of, 257; bactericidal reaction of, 257; method of testing antitoxic value of, 258.

DYSENTERY TOXIN, 254; action of, 254; components of, 255; enteric toxin in, 255; from filtration of cultures, action of, 255; nature of, 255; neurotoxin in, 255; susceptibility of various animals to, 255.

EAR, complications of cerebrospinal meningitis affecting, 551; vaccine therapy in suppurative processes of, 639.

ECHINOCOCCUS DISEASE, complement-fixation test in, 140; miostagmin reaction in, 146.

ECLAMPSIA IN PREGNANCY, normal serum in treatment of, 825.

ECZEMA, vaccine therapy for, 633.

EFFUSION in gonococcal arthritis, 579.

EHRlich, diazo reaction of, 501; on serum-fastness, 12; standard unit of diphtheria antitoxin of, 438; theory of immunization of, 7.

EHRlich AND GUTTMAN, report by, of favorable results with tuberculin, 317.

EHRlich AND WASSERMANN, method of, of standardization of diphtheria antitoxin, 437, 438.

ELLERMANN AND ERLANDSEN, cutaneous tuberculin tests of, for administration of tuberculin, 346.

ELLIOTSON, JOHN, on etiology of hay fever, 668.

EMACIATION in gonococcemia, 589.
INDEX

EMBOLISM, cerebral, in gonococcal endocarditis, 588.
EMPYEMA, complicating gonococcosis, 587; due to actinomycotic infection, treatment of, 431; due to serum treatment of pneumonia, 485; vaccine therapy in, 493.
ENDARTERITIS, syphilitic, 402; cell count of cerebrospinal fluid in, 404.
ENDOCARDITIS, complicating cerebrospinal meningitis, 553; gonococcal, see gonococcal endocarditis; streptococcus viridans cause of, 507.
ENDOTIN, 324; definition of, 326.
ENGMAN, experiments of, for acne bacillus, 624, 625; technique of, for administration of vaccine in deep-seated nodules and abscesses of acne, 629.
ENTAMERAS in the normal mouth, 635.
ENTERITIS BACILLI, group diagnosis in, 204; vaccine therapy of, 216.
EPIDEMIC CEREBROSPINAL MENINGITIS. See Cerebrospinal meningitis.
EPIDIDYMITIS, metastatic lesions following, 578; serum treatment in, 619; vaccine treatment in, 613, 614, 616.
EPITHELAN REACTION, 147; preparation of antigen in, 148; technique of, 148.
EPSTEIN on blood cultures in typhoid fever, 204.
ERDMAN on vaccine therapy in erysipelas, 637, 638.
ERYSIPelas, references on, 641; vaccine therapy in, 638, 639.
ERYTHEMA, following salvarsan injection in syphilis, 400; following treatment for hydrophobia, 738; in gonococcosis, 588.
ERYTHEMA MULTIFORME, in gonococcosis, 588.
ERYTHEMA NODOSUM, in gonococcosis, 588.
ESCHERICH, discovery of bacillus coli communis by, 232.
ESSENTIAL NEUROMAS, serum and blood treatment of, 816.
ETHER NARCOSIS for prevention of anaphylactic reaction, 100.
ETHYLHYDROCURPRESIN, use of, in pneumococcus infections, 496-499.
EXOSTOSES following gonococcal infection, 590.
EXOTOXINS, definition of, 326.
EYES, complications of meningitis affecting, 549-551; tuberculous lesions of, tuberculin treatment of, 321.
FARKAS, on allergic reactions in gonococcal infections, 601; on use of vaccine in gonococcal arthritis, 614.
FEHLING'S SOLUTION, reduction of, by syphilitic cerebrospinal fluid, 404.
FEMALE GENITAL TUBERCULOSIS, tuberculin in treatment of, 834.
FERMENTS, fibrin-dissolving, in the blood, 797.
FERMI, method of, of attenuating rabies by carbolic acid, 729.
FERRAN'S SUPERINTENSIVE METHOD OF TREATING RABIES, 731, 732.
FEVER, in gonococcal arthritis, 581; tuberculin treatment in, in tuberculous patients, 332.
FIBRIN CONTENT OF CEREBROSPINAL FLUID, 516.
FIBRIN-DISSOLVING FERMENTS IN THE BLOOD, 797.
FIBRINOGEN, in normal blood, 796; insufficiency of, 796.
FICHERA on autolyzed vaccines for cancer, 779, 780.
FIELD on absorption of toxins by the body, 456.
FINKELSTEIN AND GRUSHUN on cutaneous reactions following use of gonococcal vaccines, 602.
FISCHER on treatment of scarlet fever with neo-salvarsan injections, 647.
FITZGIBBON on vaccine treatment of vulvovaginitis, 822.
FIXED VIRUS, 719, 720.
FLEMMING, experiments of, for acne bacillus 625.
FLEXNER, experiments of, on anaphylaxis, 36; experiments of, on immunochromy of pneumococi, 13; isolation of dysentery bacillus by, 249; on serum-fastness in epidemic meningitis, 12; preparation of specific immune serum for cerebrospinal meningitis by, 521.
FLEXNER DYSENTERY BACILLUS, 249.
FOCAL INFECTION, anaphylaxis and, 171; chronic, pathology of, 169; prophylaxis of, 171; results of secondary foci in,
INDEX

167; site of focus in, 165; systemic disease and, 165; systemic diseases originating from, 167.

FOCAL INFECTION, TREATMENT OF, 171; after-management in, 174; bacteriological examination in, 174; removal of focus in, 174; vaccines and serum in, 174.

FÖCKLER, on use of vaccines in gonococcal arthritis, 614.

FOOD IDIOSYNCRASIES AND ANAPHYLAXIS, 95.

FORCE, on sensitized killed antityphoid vaccine, 187.

FRANCO-GERMAN WAR, typhoid losses of German Army in, compared with losses from other causes, 188, 189.

FRANQUÉ, on treatment of female genital tuberculosis, 938.

FRASER AND FLETCHER, investigations on bacillus leprea by, 386.

FREUND, RICHARD, on serum treatment of intoxications of pregnancy, 625.

FRIEDBERGER, on anaphylactic reaction in complement, 84; on anaphylactoid phenomena, 105.

FROMME, on allergic reactions in gonococcal infections, 601, 602.

FROMM and COLLMAN, on vaccine treatment of gonorrheal infections of female genitalia, 831.

FROST, use of vaccines by, in gonococcal arthritis, 613, 614.

FULMINATING CASES OF CEREBROSPINAL MENINGITIS, 536.

FURIOUS HYDROPHOBIA, 702.

FURIOUS RABIES, 703.

FURUNCULOSIS, staphylococcus vaccine in treatment of, 631. See also Staphylococcus vaccine.

FUSIFORM BACILLI, in acute ulcerous gingivitis, 636; in noma, 636; in Vincent’s angina, 636; infections of the mouth by, 635, 636.

FUSIFORM BACILLUS INFECTIONS, specific treatment of, 637.

GABRITSCHEWSKY, on use of streptococcus vaccines as preventive in scarlet fever, 644.

GAMPERCHT AND SINTZING, on path of tetanus toxin to central nervous system, 455.

GARRAT AND MEYER, investigations of, in unsensitized vaccines, 29.

GASTRIC ANACIDITY in pernicious anemia, 808.

GASTRIC ULCER, transfusion of blood in, 815, 816.

GASTRO-INTESTINAL SYSTEM, anaphylactic reaction in 78.

GASTROTOXIC SERUM, 761, 762.

GAY, on types of dysentery bacillus, 250; sensitized killed antityphoid vaccine of, 187.

GAY AND CLAYPOOL, on varying degrees of agglutinability of typhoid bacilli, depending on media grown in, 207, 208.

GAY AND SOUTHARD, on anaphylactic reaction, 60; on transferable sensitization in anaphylaxis, 90.

GENITAL TRACT, LOWER, infections of, complement-fixation test in, 140.

GENITO-URINARY ORGANS, TUBERCULOSIS OF, tuberculin treatment in, 322.

GERMAN LOSSES FROM TYPHOID FEVER in Franco-German War, compared with losses from other causes, 188, 189.

GERSHUN and FINKELSTEIN, on cutaneous reactions following use of gonococcal vaccines, 602.

GIBSON and BANZHAF, diphtheria antitoxin concentration method of, 436.

Gibson’s method of refining and concentrating antitoxigenic serum, 532.

GIEBISCH’S STAIN for negri bodies, 717.

GILCHRIST, experiments of, for acne bacillus, 624; on administration of vaccine in deep-seated nodules and abscesses of acne, 630.

GILLESPIE and DOCHÉZ, studies of, on antipneumococcus serum, 481, 482.

GILMAN and COCA, use of unaltered vaccines by, for cancer, 777.

GINGIVITIS, ACUTE ULCEROUS, 636, 637; treatment of, 637.

GLANDERS, 657.

GLANDULAR SYSTEM, anaphylactic reaction in, 80.

GLOBULIN CONTENT OF CEREBROSPINAL FLUID, in syphilis of central nervous system, 405; technique of determining, 417.

GLYCERIN, attenuation of rabies virus by, 729.
INDEX

Glycerin extracts of gonococcus, allergic reactions following use of, 600.
Glycosuria, in gonococcemia, 559.
"Goat-fever," 660.
Goat serum, in treatment of Graves' disease, 793.
Goat's milk in treatment of Graves' disease, 793.
Goetsch, on results with tuberculin treatment, 317.
Gonococcal arthritis, 578–581; allergic reactions following use of vaccines in, 600; complement-fixation reaction in, 603, 604, 605; diagnosis of, 578, 579, 581, 590; gonococcemia and, 583; irisitis and, 582, 616; of the spine, 580; pathology of, 579; prognosis of, 580; serum treatment of, 618, 619; stages of, 580; symptoms of, 581; vaccine treatment of, 613–615.
Gonococcal endocarditis, 584, 585; diagnosis of 590, 591; prognosis of, 584.
Gonococcal epididymitis, metastatic lesions following, 578; serum treatment in, 619; vaccine treatment in, 614, 615, 616.
Gonococcal infections, complement-fixation in, 137, see also under Complement-fixation in gonococcal infections; reactions following inoculations of vaccines in: cutaneous, 159, intracutaneous, 159, subcutaneous, 159.
Gonococcal infections of Fallopian tubes, treatment of, 831.
Gonococcal infections of female genitalia, exploratory needle in determination of etiology of, 830; general considerations of, 829; of vaccine treatment of, 829; vaccine treatment of, 830; vaginal incisions in determination of etiology of, 830.
Gonococcal iritis, 582, 583; vaccine treatment of, 616.
Gonococcal meningitis, 588.
Gonococcal ophthalmia, vaccine treatment in, 613.
Gonococcal pleurisy, 587.
Gonococcal pneumonia, 587.
Gonococcal protein, allergic reactions to, 600.
Gonococcal sepsis, 583, 585, 586; vaccine treatment of, 618.
Gonococcal septicemia, 585–587; diagnosis of, 591.
Gonococcal urethritis, vaccine treatment of, 611, 612.
Gonococcal uveitis, 616.
Gonococcal vaccines, commercial preparations of, 610; dosage of, 608, 609; preparation of, 608, 609; sensitized, 609, 610; size and intervals of inoculations of, 610, 611.
Gonococcemia, 583, 584; complications of, 587–589; remittent fever due to, 585.
Gonococcus, allergic reactions, 600–602; autolysis of, 597, 598; characteristics of, 595–597; culture media for, 591–593; examination of articular fluids for, 595; examination of blood for, 594, 595; examination of secretions for, 585; in the blood, see Gonococcemia; methods of cultivation of, 591–594; moisture required for cultivation of, 594; port of entry of, 578; serological reactions of, 602–606; temperature for cultivation of, 593, 594.
Gonococcus infections, characteristics of, lesions in, 578–589; complications of, 613; diagnosis of, 590, 591; gonococcus, 591–606; immunity in, 598–606; introduction to, 577, 578; of the mouth, vaccine therapy in, 639, 640; pathology of, 578; references on, 619–622; serum and vaccine treatment of, 608–619; serum therapy in, 618, 619; urethral, spontaneous healing of, 599; vaccine, treatment of, 611–618.
Gonococcus infections, vaccine treatment of complications of: arthritus, 613–615; immunity in, 598–606; epididymitis, 616; irisitis, 616; other complications, 608, 618; pelvic infections, 616, 617.
Gordon, W., on etiology of hay fever, 668.
Graminaceae, structural characteristics and composition of pollen of, 674.
Graminol, hay fever serum, 668; nature of, 668; results in treatment with, 689.
Graves' disease, etiology of, 759, 790; general treatment of, 792, 793; pathology of, 790, 791; serum therapy in, 793–794.
Grimme's theory of Graves' disease, 789 note.
INDEX

GRÜNAUM ON serum agglutination test for typhoid fever, 206.

GUMMATA, development of, in syphilis of the central nervous system, 402.

GUMS, diseases of, acute ulcerous gingivitis, 636, 637.

GWYN, first isolation by, of paratyphoid A and B, 211.

GYNECOLOGICAL CASES, complement-fixation test in, 140.

HAFFKINE on immunisation for typhoid fever by means of bacterial vaccinations, 179.

HAGER on results of tuberculin treatment, 317.

HALLUCINATIONS in rabies, 714.

HAMILTON on vaccine treatment of vulvovaginitis, 837.

HAMMAN, on danger of rapid reactions in tuberculin treatment, 341; on prognostic value of tuberculin hypersensitivity, 338; on tuberculin treatment of antitoxin and localized tuberculosis, 343.

HANES AND LAMBERT, on cytotomic effects of antitumor serum on tumor cells, 759.

HANSEN, discovery of bacillus leprae by, 383.

HARRIS' METHOD OF ATTENUATING RABIES, VIRUS BY RAPID DRYING, 277, 728.

HARRISON AND POLLOCK, report of, on gonococcal vaccines in English army, 611, 612.

HÄNDEL AND NEUFELD, studies of, on antipneumococcus serum, 478-481.

HAUSER, on diagnostic use of vaccine in gonococcal infection, 602; on use of vaccines in gonococcal pelvic infections, 616, 617; on vaccine treatment of gonorrhreal infection of female genitals, 834.

HAY “ASTHMA,” 684.

HAY FEVER, 665; anaphylactic action of subcutaneous injections of pollen protein in, 679; anaphylaxis and, 95; “asthma” in, 684; autumnal variety of, pollen of Dictyophelides cause of, 673, 675; bacterial theory as to cause of, 671; definition of, 672; demonstration of pollen protein in blood of, by sensitization of animals with the serum, 681; effect of pollen extract on patients subject to, 677; etiology of, 672, see also “Polletoxin”; general symptoms of, 684; history of, 665; history of etiology of, 667; immunity in, by subcutaneous injection of serum, 679; in United States, 669; local symptoms of, 683; pathogenicity of, 676; plants whose pollen cause, 673; pollen, etiological factor of, 670; sensitization in, due to resorption of pollen protein by nasal mucosa or conjunctive and spread to blood, 682, 683; specific treatment of, 684; specificity of pollen extract in, 677; summer variety of, pollen of Gramineae cause of, 673, 674; symptoms of, 675; transference of anaphylactic condition to another animal in, by inoculation with serum of sensitized animal, 679; two varieties of, 673.

HAY FEVER, ACTIVE IMMUNIZATION IN, 690; method of administration of pollen extract in, 691; preparation of alcoholic extract of pollen for, 692; preparation of saline extract of pollen for, 691; results of, 696; value of, 697.

HAY FEVER, DUNBAR'S ANTITOXIC IMMUNE SERUM FOR, 684; antitoxic value of, 685; method of use of, 686; preparation of, 684; results of treatment with, 687.

HAY FEVER, PASSIVE IMMUNIZATION IN, 684.

HAY FEVER, WEICHHARDT'S SERUM, GRAMINOL, IN, 688; nature of, 688; results in treatment with, 688.

HAYNES, method of treating hydrocephalus by sinus drainage, 544.

HEART, complications of cerebrospinal meningitis affecting, 553, 554.

HEAT, effect of, on rabies virus, 709, 710.

HEUSER, treatment of leprosy by, 392.

HEKTORN AND ROSENOW, use of autolysed vaccines by, in pneumonia, 491, 492.

HEMATURIA, ESSENTIAL, serum and blood treatment of, 814.

HEMOPHILIA, prothrombin insufficiency in, 796; serum and blood treatment of, 812.

HEMORRHAGE, anemia due to, 798, 811; blood platelets and, 797; chronic sec-
ondary anemia following, serum and blood treatment of, 811, 812; diseases with which associated, 798–802; diseases with which associated, serum and blood treatment of, 814–817; due to excess of antithrombin, 797; due to fibrin-dissolving ferments, 787; during lumbar puncture, 526; effect of serum injection on, 804; fibrinogen insufficiency and, 796; following ventricular puncture, 544; in diseases of the liver, 816, 817; in gastric and duodenal ulcer, 815, 816; in jaundice, serum and blood treatment of, 816; in pernicious anemia, 808–810; in purpura hemorrhagica, 814; in splenic anemia, serum and blood treatment of, 810; in tuberculosis, 814, 815; in typhoid fever, blood and serum treatment of, 814; physiology of clot formation, 820.


Hemorrhagic diseases, serum therapy for, 821; dosage of serum in, 822; obtaining of blood in, 821; preparation of defibrinated blood for, 821; preparation of serum for, 822; selection of donor in, 821.

Hemorrhagic Diseases of the Newborn, blood transfusion in, 812, 813, 822; defibrinated blood in therapy of, 821; serum therapy in, 822.

Hemostatic, normal serum as, 804.

Herpes labialis in gonococcal infections, 588, 591.

Hertxheimer reaction in syphilis, 400, 408.

Hesse's Medium, modified by Stokes and Hatchel, for culture of bacteria, 203.

Heterologous sensitisation of animals in anaphylaxis, 96, 97.

Hewes, gonococcus first isolated from blood by, 583.

Hyde, on induction of labor by human serum, 824.

Heymann and Moos, on use of vaccines in gonococcal pelvic infections, 617; on vaccine treatment of gonorrheal infection of female genitals, 829.

HeyneMann, on allergic reactions in gonococcal infections, 602.

Hiss, treatment of pneumonia by, with leukocyte extract, 494, 495, 496; use of leukocytic extracts by, in erysipelas, 639.

Hiss and Russell, Bacillus Y of, 249.

Hiss and Zinsser, on immunity by means of injection of leukocyte extracts, 10; treatment by, of pneumonia with leukocyte extract, 495.

Hodara, experiments of, for acne bacillus, 624.

Hodgkin's Disease, 662–664; bacteriologic findings in, 663; Coley's toxins in treatment of, 663; etiology of, 662; organism of, 662; references on, 664; sarcoma and, 663; vaccine treatment of, 663.


Högyes' method of using fresh fixed virus, 730, 731.

Homologous sensitisation of animals in anaphylaxis, 96.

Horimi, on nature of dysentery toxin, 256.

Horowitz, on toxin of Asiatic cholera, 285.

Horse serum, use of, in Graves' disease, 793; in pneumonia, 476; in Rocky mountain spotted fever, 658.

Horses, sporotrichosis in, 421.

D'Hôstalric, on results of serum treatment of plague in Annam, 274.

Hot Water Injections, in Graves' disease, 793.

Howell's Theory of Blood Coagulation, 797.

Huber and Blumenthal, on convalescent scarlet fever serum therapy, 642.

Hunt, on serum agglutination test for typhoid fever, 207; on types of paratyphoid fever, 212.

HutinsoN, on intraspinal injection of Doper antin meningitis serum, 94.

Hydrocele fluid, in preparation of culture media for gonococcus, 592.

Hydrocephalus, effect of antin meningitis serum on, 562; following meningitis, 549; in cerebrospinal meningitis, 536, 537, 540; mild treatment of, 541, 542; treatment of, 539, 540, 559; treatment of, in posterior basic meningitis, 543.
INDEX

Hydrochloric acid, in treatment of gastric acidity in pernicious anemia, 506.

Hydrogen peroxide, in treatment of ulcerous gingivitis, 637; prophylactic use of, in meningitis epidemics, 506, 575.


Hydrotherapy in Graves' disease, 793.

Hyoscin in treatment of cerebrospinal meningitis, 548.

Hyperacousis in rabies, 714.

Hyperkeratosis gonoemphoica, 588.

Hyperplasia of the bone marrow in pernicious anemia, 808.

Hyperthyroidism, Graves' disease and, 789.

Hysteria, differentiation of, from rabies, 716.

Ice, in treatment of cerebrospinal meningitis, 548.

Ice-bag, use of, in Graves' disease, 792.

Icterus, calcium content of the blood and, 796; excess of antithrombin in, 797; in gonococcal, 589.

Imbecility, following meningitis, 549.

Immune bodies, formation of, following meningococcus vaccination, 572; in blood of patients recovering from meningitis, 569; in blood of patients vaccinated for meningitis, 570, 571; in sporotrichosis, 423; production of, by sera and vaccines, 567, 568.

Immune serum, alien, duration of immunity with injection of, 9; as carrier of specific chemical therapeutic agent, 14; frequent unfavorable action of, 15; homologous, duration of immunity with injection of, 9.

Immune serum of dysentery, 257; production of, 259.

Immune streptococcus opsonin, production of, 649.

Immune-ferment reactions, 141; in serodiagnosis of pregnancy, 142.

Immunity, by injection of albuminous acids derived from proteolysis of bacterial cells, 11; by injection of leukocyte extracts, 10; chemical nature of, 1; chemical reactions between invading organism and host in, 15; conferred by antitphoid vaccine, duration of, 196; development of, 1; development of, in syphilis, 398; duration of, after meningococcus vaccination, 573; duration of, relative to proportion of toxin and antitoxin in immune sera, 9; duration of, with homologues and alien sera, 9; duration of passive and active, 9; immunological reactions in, 2; in erysipelas, 638; in gonococcal infections, 598–605; in sporotrichosis, 423; in typhoid fever, by vaccine inoculations, 178; invading organism in, 11; of mouth tissues, 635; produced by antirabic treatment, 738; produced by final digestion of toxic bacterial protein by body ferment, 202; relation of host to invading organism in, 6; relative duration of, with subcutaneous and intravenous injections, 9; specific chemoserological therapy in, 13; specific deference of host in, 2; specific therapy in, 2; to actinomycosis, 431; to blastomycosis, 426; to Malta fever, 660; to meningitis, production of, by vaccination, 569; to pneumonia, experimental studies on, 473–476.

Immunization, active. See Vaccine therapy, in blastomycosis, 426; against Malta fever, 660; antibodies in, 6; antitoxins in large doses in, 8; by killed cultures, against glands, 657; duration of passive and active immunity in, 9; Ehrlich's theory of, 7; in ear and eye infections, 639.

Immunization, active, in infectious diseases, 19; clinical evidence for and against, 25; conditions favorable to, 19; dosage of serum in, 20; effect of, on antibodies other than opsonins, 24; in general infections, 26; in localized infections, 26; in not strictly localized infections, summary of, 27; neglect of surgical procedures for, 28; opsonin curve in, 19; reactions following, 28; relation of opsonins in, to recovery, 21;
relation of opsonins to recovery in, in generalized infections, 22; relation of opsonins to recovery in, in localized infections, 21; relation of opsonins to recovery in, in not strictly localized infections, 23; sensitized vaccines in, 28.

Immunization, passive. See Serum therapy.

Immunological reactions, as physico-chemical processes, 3; chemical nature of antigens in, 4; grouping of, 5; in diagnosis, 117.

Inanition, anemia due to, 799.

Infecting organisms, defense of host against, 6; formation of antibodies in host upon invasion of, 6; relation of host to, 6.

Infection, mode of, in actinomycosis, 429, 430; in Malta fever, 659; in sporotrichosis, 421, 423.

Infectious diseases, Abderhalden's theories of cause of, based on relations of invading organism to host, 15; active immunization in, 19; condition of serum favoring active immunization in, 19; non-specific cause of symptoms of infection in, 18; surgical procedures in, 28; treatment of, by intravenous injections of foreign proteins, 31; Vaughan's theory of proteolysis of bacterial proteins as cause of, 16.

Infectious diseases, vaccine therapy in, 19; clinical evidence for and against, 25; clinical evidence in localized infections in, 26; clinical evidence in not strictly localized infections in, summary of, 27; dosage of serum in, 20; effect of, on antibodies other than opsonins, 24; neglect of surgical procedures for, 28; opsonic curve in, 19; reactions following, 28; relation of opsonins in, to recovery, 21; relation of opsonins in, to recovery in generalized infections, 22; relation of opsonins in, to recovery in localized infections, 21; relation of opsonins in, to recovery in not strictly localized infections, 23; sensitized vaccines in, 28.

Influenza meningitis, bacteriological examination of cerebrospinal fluid in, 518.

Iodid of potassium, use of, in actinomycosis, 431; in blastomycosis, 426.

Iodid, in treatment of cerebrospinal meningitis, 548.

Iodin, prophylactic use of, in meningitis epidemics, 566.

Iodin in the thyroid gland, decrease of, in Graves' disease, 791.

Iodin tuberculin, 326.

Insanity, immune-ferment reaction in, 145.

Intestinal antiseptics in treatment of acute typhoid carrier, 222.

Intestine, involvement of, in Graves' disease, 791.

Intoxications, anemia due to, 799.

Intoxications of pregnancy, antistreptococcus serum in, 826; normal horse serum in treatment of, 825.

Intracutaneous gonococcal reactions, 159.

Intracutaneous tuberculin reaction, 157.

Intraneural injections of antitoxin, in tetanus, 462.

Intraspinai injections of antitoxin, in tetanus, 462.

Intraspinous injection of salvarsan and neosalvarsan, 409, 410.

Intraspinous injection of serum containing salvarsan in syphilis of central nervous system, 410-417; after-treatment of, 418; technique of, 414-418.

Intravenous injection of antitoxin, rapidity of effect of, 9; of saline solution in treatment of Asiatic cholera, 293.


Intravenous injection of salvarsan, length of treatment by, 419.

Intravenous and subcutaneous injection of antitoxin, comparative duration of immunity in, 9.

Intraventricular puncture, in posterior basic meningitis, 561.

Iriss, gonococcal, 582, 583, 616; recurrences of, 582.

Iron, use of, in cerebrospinal meningitis, 548; in Graves' disease, 792.

Iron tuberculin, 324.

Irons on subcutaneous gonococcal reactions, 159.

Ishigami, tuberculin of, 326.
INDEX

Iso-antibodies, cytolytic effects of, 766-767; in experimental cancer in vitro, 767, 768; specificity of, 766.
Iso-lysins, action of, in experimental cancer in vivo, 768, 769.
Itching in serum sickness complicating cerebrospinal meningitis, 555.

Janin, experiments of, in treatment of leprosy with serum prepared with nodules of patient, 388.
Jaundice, hemorrhage in, serum and blood treatment of, 818.
Jensen, experiments of, with a hetero- genetic tumor antisera, 762.
Jessen's tuberculin, 324.
Josling and Bull, on anaphylactic symptoms produced by injections of washed typhoid bacilli, 201.
Jochmann, preparation of specific immune serum for cerebrospinal meningitis by, 521.
Jochmann's albumose-free tuberculin, 330; preparation of, 324.
Joints, complications of cerebrospinal meningitis affecting, 540, 541; gonococcal involvement of, see Gonococcal arthritis; localization of Malta fever infection in, 661.
June cold, pollen of Graminaceae, cause of, 673, 674.

KaoLin, local use of, in removing diphtheria bacilli from surfaces of mucous membranes, 450.
Karkinsh, isolation by, from leprosy cases, of acid-fast bacilli, 383.
Keene's operation, for posterior basic meningitis, 543.
Kidney, anaphylactic reaction in, 80; inflammation of, caused by colon bacillus, 239; involvement of, in gonorrhea, 587, 588; malformation or displacement of, predisposing to colon bacillus infection, 239; operative procedures predisposing to colon bacillus infection in, 240.
Kimpton's method of indirect transfusion of blood, 807.
Kiralyfi on bacteriological tests of cultures of typhoid bacillus obtained from bile in typhoid fever, 204.

Kitasato, results of serum treatment of plague in Formosa, 274.
Klaue on vaccine treatment of epididymitis, 616.
Klebs, discovery of diphtheria bacillus by, 434.
Klein and M'Donagh, use of sensitized vaccines by, 609, 611.
Klemperer, on immunity to pneumococcus, 472, 473.
Koch, on antityphoid vaccination in German Army, 182; on necessity of tuberculin reaction, 340; on original use of tuberculin in treatment, 333; on treatment of scarlet fever with scarlet fever convalescent serum, 643; subcutaneous tuberculin test of, 151; tuberculin of, principle of, 327.
Koch's Baccilli-emulsion, 330; preparation of, 324.
Koch's Original or Old Tuberculin, 330; preparation of, 323.
Koch's Tuberculin-residue or New Tuberculin, 330; in continuous minimal dosage in tuberculin treatment, 333; preparation of, 324.
Kocher's operation, in posterior basic meningitis, 543.
Koessler's method of active immunisation in hay fever, 691.
Kolaczek, on treatment of acute phlegmons and abscesses by injection of albuminous acids derived from proteolysis of bacterial cells, 11.
Kolle and Martini, investigations of, on bactericidal properties of plague immune serum, 267.
Kolle and Strong, investigations of, on antibacterial action of plague immune serum, 268.
Kolle and Wassermann, preparation of specific immune serum for cerebrospinal meningitis by, 521.
Kolle's cholera serum, results with, 292.
Kraus, cholera serum of, results with, 291, 292; on ophthalmic diagnostic reaction in typhoid, 209; production of cholera toxin by, 284; production of serum by, for treatment of Asiatic cholera, 284.
Kraus, Hober and Ishitara, experimentation of, on the bacillus of leprosy, 384.
Kremer on results covering tuberculin bacilli in sputum following tuberculin treatment, 320.
Kreucker on typhoid agglutination tests in tuberculosis, 211.
Kritschewsky and Bierger, experimentation of, on the bacillus of leprosy, 384.
Kruse, finding of dysentery bacillus by, 249.
Külbs, findings of, in gonococcal endocarditis, 584.
Kutner and Schwenke, on allergic reactions in gonococcal infections, 601; on vaccine treatment of gonococcal arthritis, 613.
Kyes, separation of hemolytic and neurotoxic constituents of cobra venom by, 744.

Lange, induction of, human serum in, 826.
Lachman glands, anaphylactic reaction in, 80.
Lactic acid bacilli, therapeutic use of, in vaginitis, 833.
Lamar, on alteration of biologic and cultural characteristics of pneumococcus by variation of media and environment, 204; studies of, on soap, serum, and boric acid mixtures in pneumococcus infections, 500.
Lambert and Hanes, on cytolytic effects of antitumor sera on tumor cells, 759.
Landmann's Tuberculol, preparation of, 325.
Landsteiner, experiments of, on effect of immune serum on spermatozoa, 758; on immunity reactions, 5.
Landsteiner, Mueller and Postel on alcoholic extracts of non-syphilitic liver in Wassermann reaction, 128.
Lance, colloidal gold reaction of, 3; diagnostic differentiation by means of, 4; in syphilis of central nervous system, 407.
Langebach and Wolff, report by, of favorable results with tuberculin, 317.
Laryngitis, tuberculous, tuberculin treatment of, results in, 321.
Laverde, experiments of, in treatment of leprosy with serum from animal inoculations, 387.
Leber and Steinhardt's tuberculin, 324.

Lerredo, Coca, and Dorsane, report of, on vaccine therapy in cancer, 777, 778.
Leishman, Col. Sir Wm. B., explanation of, unfavorable results in typhoid immunization in British Army in Boer War, 181; on typhoid immunization by vaccine inoculation, 179.
Letter coll, use of, in Graves' disease, 792.
Lenartowicz on complement-fixation reaction in gonococcal infections, 605.
Lenze, on pathology of rabies, 708.
Leptra toxin, prepared from cultures of leprosy bacillus in treatment of leprosy, 391.
"Leprolin" of Rost in treatment of leprosy, 389.
Lepromata, tissue juice of, in treatment of leprosy, 389.
Leprosy, bacillus of, uncertainty of successful cultivation of, 385; etiology of, 384; micro-organisms cultivated from cases of, classification of, 383; natin and nasin benzoyl in treatment of, 385; non-specific vaccine treatment of, 394; "self-limited," 395; serological tests for etiological bacillus of, 384; serum treatment of, 387; spontaneous cure and improvement in, 394; streptothrix in, in relation to treatment, 385; treatment of, with natin, 392; tuberculin in treatment of, 394; vaccine treatment of, 389; varieties of: acid-fast bacilli which do not produce colonies, 383; acid-fast organisms which produce yellow or orange-colored colonies, 383; acid-fast streptothraces, 383; anaerobic acid-fast organisms, 383; partially acid-fast or acid-resistant diphtheroid organisms, 383.
Leprosy bacilli, chloroform and alcohol extraction of fatty substances of, for treatment of leprosy, 391; killed, prepared from leprous nodules and surrounding tissue, in treatment of leprosy, 389; killed, suspension of, in treatment of leprosy, 391; live cultures of acid-fast variety of, in normal saline solution in treatment of leprosy, 388; living cultures of, suspended in saline solution, in treatment of leprosy, 391; sensitized killed cultures of, in treatment of leprosy, 391.
INDEX

Leprosy streptothrix vaccine of Williams in treatment of leprosy, 391.
Leukemia, excess of antithrombin in, 797; serum treatment of hemorrhages in, 819; see also Hodgkin's disease.
Leukocytic extracts, in treatment of erysipelas, 639; in treatment of pneumococcus infections, 494-496.
Leukocytosis, in gonococcal infection, 589; in gonococcal septicemia, 591.
Leukopenia, in Graves' disease, 791.
Von Leyden, on convalescent scarlet fever serum therapy, 643.
Von Leyden's use of unaltered vaccines for cancer, 775, 776.
Lindeman's method of indirect transfusion of blood, 807.
Liston, acid-fast streptothricides isolated in leprosy cases by, 381.
Liver, anaphylactic reaction in, 81.
Liver, diseases of, excess of antithrombin in, 797; fibrinogen insufficiency in, 796; transfusion of blood in, 816, 817.
Liver, involvement of, in gonococceemia, 589.
Loeffler, first to cultivate diphtheria bacillus, 434.
Lovely on treatment of acne cases with vaccine, 631.
Lowenstein, method of, of administering rapidly progressing doses of tuberculin, to reach high doses in shortest time, 341; on results covering tubercle bacilli in sputum following tuberculin treatment, 320.
Lürke and Orndechiew, experiments of, on relative duration of immunity with homologous and alien sera, 9.
Leutin reaction in syphilis, 160; diagnostic value of, 162; negative, 160; positive, 160; preparation of antigen in, 160; result of treatment on, 162; technique of, 160.
Lumbar puncture, accidents during, 526; administration of cerebrospinal fluid by, 522-523; and injection of serum in cerebrospinal syphilis, 415-417; needle for, 525; posture of patient for, 525; route of, 525; site of, 524, 525; technique of, 524, 525.
Lungs, involvement of, in gonorrhea, 587.
Luttiger on vaccine treatment of whooping cough, 301.
Lymph, anaphylactic reaction in, 85.
Lymph glands, involvement of, in Graves' disease, 791.
Lymph nodes, in gonococcal infection, 599; in Hodgkin's disease, 662.
Lymphocytosis, in Graves' disease, 791.
Lymphogranulomatosis, 662.
Lyssa. See Hydrophobia.
Lyssophobia, 702; differentiation of, from rabies, 716.

MacCulloch, John, on etiology of hay fever, 608.
MacFaydan, production of cholera toxin by, 283.
MacNeal and Chace on finding bacillus typhosus in duodenal fluid in relapse, 204.
MacRae needle for obtaining blood for serum treatment of syphilis, 414.
Madsen on mass action in passive immunity, 8.
Mabura foot, 432; black variety of, 432; morphology of, 432; pale variety of, 432; resemblance of, to actinomyces, 432.
Magendi, experiments of, on anaphylaxis, 36.
Malignant pustule, treatment of, 655.
Mallein in treatment of glands, 657.
Mallow and Horners, investigations of whooping-cough by, 296.
Malta fever, agglutination reaction in, 124; diagnosis of, 660, 661; etiology of, 659; immunity to, 660; mode of infection in, 659; occurrence of, 659; prophylactic inoculation against, 661; references on, 661; serum treatment of, 661; vaccine treatment of, 661.
Mantaux, intracutaneous tuberculin reaction of, 157; intracutaneous tuberculin test of, for administration of tuberculin, 347.
Manwaring on protective powers of injection of leukocyte extracts, 10.
MARIE AND MORAX, on path of tetanus toxin to central nervous system, 455.
MARIE'S method of preparing antirabic serum, 735.
MARIE'S method of serum-vaccine treatment for rabies, 733.
MARINE'S theory of Graves' disease, 790.
MARL, on method of destruction of plague bacilli by immune serum in relation to their virulence, 267.
MARMOREK, work of, on antistreptococcus serum, 650.
MARTINI AND LENTZ, on types of dysentery bacilli, 250.
MASON, in passive immunity, 8.
 Massage, in treatment of Graves' disease, 793; prostatic, in gonococcal infections, 607.
McNEIL AND SCHWARTZ, studies of, on complement-fixation in gonococcal infections, 604-606.
MDONALD AND KLEIN, on vaccine treatment of gonococcal infections, 615; use of sensitized gonococcal vaccines by, 609, 611.
MEDITERRANEAN FEVER. See also Malta fever.
MENINGISMUS, 513, 514.
MENINGITIS, Besredka's method in treatment of, 102; cerebrospinal, see Cerebrospinal meningitis; epidemic, precipitin diagnostic test for, 125; Netter on treatment of, 102; Weil on treatment of, 102.
MENINGITIS, INTRASPINAL, Besredka's treatment of, 102; in doubtful diagnosis, 102; in undoubted diagnosis, 102; symptomatic, 102.
MENINGITIS, TUBERCULOUS, tuberculin treatment in, 322.
MENINGO-ARTHR-O-MYELITIS, 402.
MENINGOCOCCUS, differentiation of, from gonococcus, 596, 597; examination for presence of, 565; strains of, 561.
MENINGOCOCCUS CARRIERS, 553, 564.
MENINGOCOCCUS INFECTIONS, gonocococcus infections compared with, 618.
MENINGOCOCCUS MENINGITIS, bacterial examination of cerebrospinal fluid in, 518.
MENINGOCOCCUS SEUM. See Antimeningitis serum.
MENINGOCOCCUS VACCINATION, clinical reaction to, 571, 572; preparation of, 570, 572, 573, 577; prophylactic, 569, 570, 572, 575.
MENINGOMYELO-ENCEPHALITIS, 549.
MENTALITY, changes in, following meningitis, 549.
MENTHAL, use of, in serum sickness, 558.
MERCURIAL treatment in syphilis, influence of, on Wassermann reaction, 135.
MERCURIALIZED SERUM, in treatment of syphilis of central nervous system, 415.
MERCURY, in treatment of cerebrospinal syphilis, 408, 409, 418; in treatment of scarlet fever, 647.
METATHROMBIN, 804.
METALNIKOFF, experiments of, on effect of immune sera on spermatozoa, 758.
METASTATIC PROCESSES, alveolar abscess and, 635, 636; pyorrhea and, 635.
METALNIKOFF on treatment of leprosy with serum, 387.
METALNIKOFF and Besredka, investigations of, in typhoid immunisation, 186; sensitized living anti-typhoid vaccine of, 186.
METALNIKOFF, ROUX AND SALIMBENI, investigations of, on cholera toxin, 281; production of cholera toxin by, in artificial media, 282.
METRORRHAGIA, case of, 814; serum treatment of, 819.
MEYER AND RANSOM on path of tetanus toxin to central nervous system, 455.
MEYER-BUPPEL, tuberculin-serovaccine of, 324.
MICROCoccus aureus INFECTIONS, serum therapy in, 654.
MICROCoccus catarrhalis, differentiation of, from gonococcus, 597.
MICROCoccus melitensis, 659.
MIDDLE EAR suppuration complicating cerebrospinal meningitis, 551.
MILK, in treatment of Graves' disease, 792.
Milk of thyroidectomized goats, in treatment of Graves' disease, 793.
MINETT, treatment of leprosy by, with nastin, 393.
MIOSTAGMIN REACTION IN CARCINOMA, 145; preparation of antigen in, 146; technique of, 146; value of, 146.
INDEX

MIOSTAGMIN REACTION IN TUBERCULOSIS, 146.
MIXED VACCINES, in treatment of secondary infections of the mouth, 639, 640.
MORBIDUS' THEORY OF GRAVES' DISEASE, 783.
MOELLER, on results of tuberculin treatment at Belfig sanatorium, 317.
MONILIA CANDIDA, 424. See also Oidium albicans.
"MONORECIDIV," 400.
MOORE AND NOUCHI, discovery by, of treponema pallidum in paresis, 402.
MOOS AND HEYMANN, on use of vaccines in gonococcal pelvic infections, 617.
MORBUS MACULOSUS NEONATORUM, 812, 813; serum and blood treatment of, 813.
MORGAN, on types of dysentery bacilli, 251.
MORGENSETH, chemotherapy of pneumococcus infections of, 14; studies of, on chemotherapy in pneumococcus infections, 496, 497.
MORO, tuberculin saline reaction of, 157.
MORPHIN, use of, in cerebrospinal meningitis, 548; in serum sickness, 558.
MORRIS AND DOKE, on treatment of acne cases with vaccine, 630.
MOSER, on use of antistreptococcus serum in scarlet fever, 646; scarlet-fever streptococcus serum of, 650.
MOSQUITO, as source of infection in Malta fever, 659.
MOUTH, INFECTIONS OF, acute ulcerous gingivitis, 636, 637; by fusiform bacilli and spirochetes, 635, 636; references on, 641.
MOUTH, SECONDARY INFECTIONS OF, mixed vaccines in treatment of, 639; vaccine therapy in, 639.
MUCOUS MEMBRANES, susceptibility of, to gonococcal infection, 558.
MUELLER AND OPPENHEIMER, on complement-fixation in gonococcal infections, 137.
MUIR AND RITCHIE, on agglutination and immunity in typhoid fever, 208.
MULTIPLE INFECTIONS, co-operating aggressive action of, on host, 15; co-operating favorable action on, 15.
MULTIPLE NEURITIS, in gonococcal infection, 588.

MULZER AND UHLENHUTH, studies of, on the infectiousness of the blood in syphilis, 388.
MURREL, use of autogenous vaccines by, in gonococcal arthritis, 613.
MUSCULAR SYSTEM, anaphylactic reaction in, 75.
MYOSITIS, polyvalent streptococcus horse serum in, 176; vaccine therapy for, 175.

NAGEL, on results of tuberculin treatment at sanatorium at Cottbus, 318.
NASTIN, in treatment of leprosy, 385, 392.
NASTIN BENSOYI, injection of, in treatment of leprosy, 385.
NEGATIVE PHASE AFTER MENINGOCOCCUS VACCINATION, 572, 575.
NEPHI BODIES, 704, 710, 711; morphology of, 710, 711; spread method of demonstrating, 716, 717.
NEISSER AND SHIGA, demonstration of action of toxin of dysentery bacilli by, 254.
NELIS AND VAN GEUCHTEN, discoveries of, in rabies, 708.
NEOSALVARSAN in scarlet fever, 647; intraspinous injection of, in syphilis of the central nervous system, 410; relative value of salvarsan and, 409.
NEPHRITIS, GONOCOCCAL, 589.
NEPHROTOXIN, 761; specificity of, 760.
"NERVOUS SYSTEM, anaphylactic reaction in, 85; involvement of, in gonococcosis, 589; syphilis of, see Syphilis.
NETTER, on treatment of meningitis, 102.
NEU, on treatment of female genital and peritoneal tuberculosis, 834; on vaccine treatment of gonorrhreal infection of female genitals, 832.
NEUFELD, dilution method of, in estimating opsonic index in blood serum following antityphoid vaccination, 186.
NEUFELD AND HÄNDEL, studies of, on antipneumococcus serum, 478-481.
NEURITIS in gonococcal infection, 588.
NEUROCYTES HYDROPHOBIC, 710, 711.
"NEURORECIDIV," 400.
NEUROTOXIC SERUM, 760.
NICHOLLS, on treatment of leprosy with killed bacilli prepared from leprous nodules and surrounding tissue, 389.
NICOLAIS AND CONSEIL, attempted immu-
INDEX

IZATION of typhoid patients by, with blood serum of convalescents, 227.
Nicolle and Blaizot, method of, of preparing gonococcal vaccines, 608, 609.
Nocardia, 426.
Noguchi, 
Noguchi reaction, 160; modification of Wassermann test for syphilis by, 130; on nature of Wassermann reaction, 133; on preparation of acetone insoluble antigen for Wassermann reaction, 128; on “proteotropic complement-fixation,” 128.
Noguchi human cell reaction for syphilis, 130; with heated serum, 130.
Noguchi reaction, 417.
Noguchi test for globulin, 405, 417, 418, 517.
Noguchi and Moore, discovery of treponema pallidum by, in parasis, 402.
Noma, fusiform bacilli in, 636.
Nonne test of cerebrospinal fluid, 516, 517.

Noum-Apel method of determining globulin content of cerebrospinal fluid, 418.

Noon and Freeman, experiments of, on active immunization in hay fever by hypodermic inoculation of pollen vaccine, 690, 691.

Normal serum, action of, 804, 805; administration of, 805; in treatment of anemia and hemorrhagic diseases, 802-805; in treatment of arthritic purpura, 814; in treatment of chronic secondary anemia following hemorrhage, 811; in treatment of essential hematuria, 814; in treatment of hemophilia, 812; in treatment of hemorrhage, in jaundice, 816; in leukemia, 810; in treatment of metorrhagia, 817; in treatment of morbus maculosis neonatorum, 813; in treatment of pernicious anemia, 810; in treatment of purpura hemorrhagica, 814; method of obtaining, from human beings, 803, 804; method of obtaining, from rabbit, 803; properties of, 804; in splenic anemia, 810; in typhoid fever, 814.

Nucleoproteins, specificity of, 756, 757.
Nutrition in treatment of cerebrospinal meningitis, 547.
Nystagmus in rabies, 714.

Ohna, on types of dysentery bacilli, 250.
Oidiodyctos, 424-426.
Oidium albacans, 424; morphology of, 424; pathogenesis of, 424.
Ophthalmia, gonococcal, vaccine treatment in, 613.
Ophthalmic diagnostic reaction in typhoid, 209.
Ophthalmo-tuberculin reaction, 158.
Opie, on protective powers of injection of leukocyte extracts, 10.

Optonic curve in active immunization, 19; effect of large inoculations in, 20; effect of small inoculations in, 20.

Optonic index, bacterial suspension in estimation of, 149; definition of, 19; dilution method of estimating, in blood serum following antityphoid vaccination, 186; in diagnosis of gonococcal infections, 603; in vaccine therapy of cutaneous diseases, 628; leukocyte suspension in estimation of, 149; technique of estimation of, 149.

Optonic reaction in diagnosis, 149; clinical value of, 150; significance of, 149; technique of, 149.

Optonins, relation of, to recovery from disease, 21; in generalized infections, 22; in localized infections, 21; in not strictly localized infections, 23.

Optonins in gonococcal infection, 603

Optonins in serum, relation of phagocytosis to, 19.

Optic atrophy, salvansan and, 408.
Optic nerve, structure of, 549, 550.
Optochin, action of, against pneumococcus, 497; in treatment of lobar pneumonia, 499; in treatment of ulcer serpens, 502; toxic symptoms due to, 500.

Organ affinity, definition of, 11.
Organ antisera, intravital action of, 759-761.

Osler, on immunity with typhoid vaccine, 178.

Otitis media, vaccine therapy in, 630.
Otto, experiments of, on anaphylaxis, 38; on antianaphylaxis, 98; on transferable sensitization in anaphylaxis, 98.

Ozytuberculin, 326.

Palladock, experiments of, in treatment of leprosy with complement-containing
INDEX

serum of animals together with salvarsan, 388.

PALSIES, eye, complicating cerebrospinal meningitis, 550.

PANCREAS, anaphylactic reaction in, 80.

PANE’S SERUM FOR PNEUMONIA, 476.

PANICHI AND TIZZONI, preparation of anti-pneumococcus serum by, 477.

PANOPHthalmitis complicating cerebrospinal meningitis, 550.

PAPÉE, on use of arthigon in gonococcal arthritis, 614.

PARACOLON BACILLI, association of, with bacillus coli, 235.

PARALYSIS, complicating meningitis, 549; following antirabic treatment, 739–741; following rabies, causes of, 740; in rabies in dogs, 713, 714; in rabies in man, 715; syphilitic spinal ependymal, cell count of cerebrospinal fluid in, 404.

PARALYTIC HYDROPHOBIA, in man, 715.

PARALYTIC RABIES. See Dumb rabies.

PARAMENINGOCOCCUS, 557.

PARASYPLELIS, 403.

PARATYPHOID BACILLI, association of, with bacillus coli, 235.

PARATYPHOID FEVER, 211; analysis of results in bacterial therapy of, 216; bacterial therapy of, 216; discussion of results in bacterial therapy of, 218; organisms of, 211; symptoms of, 213; types of, 212.

PARATYPHOID INFECTIONS, agglutination reaction in, 124.

PARATYPHOUS A AND B, characteristics of, 211; occurrence of, 211.

PARRIS, 403; cell count of cerebrospinal fluid in, 405; general, value of Wassermann test in, 134; globulin content of cerebrospinal fluid in, 405; infectiousness of blood in, 398; intraspinal use of neosalvarsan in, 410; salvarsanized serum for intraspinal treatment of, 14; serum for therapy in, 411; treponema pallidum in, 402; use of salvarsan in, 408; Wassermann reaction in cerebrospinal fluid in, 407.

PARK on types of dysentery bacilli, 250.

PASSIVE ANAPHYLAXIS. See Anaphylaxis, passive.

PASSIVE IMMUNIZATION. See Serum therapy.

PASTEUR, discovery of pneumococcus by, 468; experiments of, in rabies, 703.

PASTEUR TREATMENT FOR RABIES, 722–728.

PEARCE on vaccine therapy in obscure diseases, 639.

PEIPER, treatment of leprosy by, with natin, 393.

PELLAGRA, transfusion of blood in, 815.

PELVIC INFECTIONS, complement-fixation test in, 140; gonococcal, vaccine treatment of, 612, 613.

PEPTONE FOR PREVENTION OF ANAPHYLACTIC REACTION, 100.

PERCUTANEOUS TUBERCULIN REACTION, 157.

PERIARTHritis. See also Arthritis; gonococcal, 579.

PERICARDITIS, accompanying gonococcal endocarditis, 584, 587; complicating gonococcosis, 587.

PERICARDIUM, involvement of, in gonococcosis, 587.

PERIPHERAL NERVOUS SYSTEM, anaphylactic reaction caused by, 88.

PERITONITIS, in gonococcal infection, 589; tuberculous, tuberculin treatment in, 322.

PERITUBERCULAR ABSCES, metastatic lesions following, 578.

PERIVASCULARIS in syphilis, 402.

PERNICIOUS ANEMIA, diagnosis of, 808; treatment of, by transfusion of blood, 809, 810; treatment of hemorrhage in, 810.

PERNICIOUS VOMITING OF PREGNANCY, normal serum in treatment of, 825.

PERTUSSIS. See Whooping-cough.

PETECHIE, in gonococcal endocarditis, 584, 585, 588.

PETRUSCHEXY on results with tuberculin treatment, 317.

PETIT AND BIEROY, on specificity of nucleoproteins, 756.

PFIFFER, investigations of, on immunity in typhoid fever, 178; on nature of cholera toxin, 280, 281; on “temperature drop” in anaphylaxis, 86.

PFIFFER AND DIEUBONNE on bactericidal properties of plague immune serum, 266.

PFIFFER AND KOLE, investigations of, on immunization in typhoid fever, 179.

PHAGOCYTOSIS, relation of, to opsonic content of serum, 19.
Pharmacetin, use of, following intraspunous injection of serum in cerebrospinal syphilis, 418; in cerebrospinal meningitis, 548.

Pheuric on results covering tuberculin bacilli in sputum following tuberculin treatment, 330.

Phosphoria in rabies, 714.

Pick, experiments of, with antitens serum, 750, 760; on alteration of antigenic qualities of albumins, 5; on formation of antigens, 4.

Pickert and Löwenstein, views of, on tuberculin action, 328.

v. Piqurt, cutaneous tuberculin test of, 155; on existence of condition of sensitization in typhoid, 310.

v. Piqurt and Schick, experiments of, on anaphylaxis, 37; on anaphylactic sensitization of man, 41; symptomatic treatment of serum disease of, 103.

Plague, agglutination reaction in, 125; serum treatment of, 266; vaccination in, as prophylactic measure, 266; vaccine therapy in, 266.

Plague antitoxic sera, 278.

Plague immune serum, 206; antibacterial action of, 268; bactericidal properties of, 266; bactericidal reaction of, 266; method of testing immunizing value of, 277; multivalent, 278; opsonic action of, 271; phagocytosis of leukocytes in action of, 270; result of treatment with, in animals, 271; result of treatment with, in man, 272; selection of, 277; specific immunizing properties of, 266; varieties of, 278.

Pleocytosis, in cerebrospinal fluid, syphilis of central nervous system, 404, 405.

Pleurisy, involvement of, in gonorrhea, 587.

Pleurisy, gonococcal, 587.

Pneumococci, specific chemotherapeutical treatment in, 13.

Pneumococcus, alteration of biologic and cultural characteristics of, by variation of media and environment, 204; determination of type of, 483, 484; discovery of, 468; immuno-chemistry of, 13; occurrence of, 468, 469; types of, 481-483; ulcus cornos serpens due to, 502, 503.

Pneumococcus infections, chemotherapy in, 460-500; general considerations of, 468-473; references on, 503-505; serum therapy in, 473-487; ulcus cornos serpens, 502, 503; use of leukocyte extract in, 494-496; vaccine therapy in, 487-494.

Pneumococcus meningitis, specific chemotherapeutical therapy of, 14; use of soap, serum, and boric acid mixture in, 501.

Pneumococcus vaccine, results of use of, 469-491.

Pneumonia, acute lobar, 469; course of, 472; determination of organism causing, 478, 484; effects of serum therapy in, 484; etiology of, 470; methods of treatment of, 471, 472; occurrence of, 469, 470; organisms causing, 482; pathology of, 471; production of antibodies in, 472; treatment of, by specific antitoxin, 480; use of leukocyte extract in, 494-496; vaccine therapy in, 487-494.

Pneumonia, gonococcal, 587.

Pneumonia, septic, complicating cerebrospinal meningitis, 553.

Pneumonic plague, serum treatment in, 276.

Pollantin, Dunbar's antitoxic immune serum for hay fever, 684; determination of antitoxic value of, 685; method of use of, 686; preparation of, 684; results of treatment with, 687; varieties of manufacture of, 686.

Pollen, etiological factor of hay fever, 670; relation between quantity of, in atmosphere and intensity of symptoms, 671.

Polleren antitoxin, effects of subcutaneous injections of, 679.

Pollen disease, 665.

Pollen extract for active immunisation in hay fever, 691; alcoholic preparation of, 692; method of administration of, 692; precautions as to, 697; results of, 696; saline preparations of, 691; value of, 697.

Pollen protein, demonstration of, in blood of hay fever, by sensitisation of animals with the serum, 681. "Pollentoxin," 677; anaphylactic action
INDEX

of subcutaneous injections of, in hay fever, 679; effect of, on hay fever sub-
jects, 677; is it a true toxin? 678; na-
ture and composition of, 677; sensitiza-
tion caused by parenteral digestion of
proteins in, in hay fever, 679; specific-
ity of, 677.

Pollock and Harrison, report of, on
gonococcal vaccines in English army,
612.

Polymorphonuclear Leukocytosis in gon-
ococcal infection, 589.

Polyvalent serum, Börner's, 477, 478; in
treatment of ulcer cornes serpens, 502.

Polyvalent vaccines, gonococcal, 608; in
epididymitis, 616; in gonococcal ar-
thritis, 614.

Poor and Steinhardt on rabies virus,
709.

Portis, experiments of, with serum in
Graves' disease, 793, 794.

Posterior basic cerebrospinal meningi-
tis, 553; treatment of, 542–545, 561.

Pottravine on Asiatic cholera toxin, 285.

Precipitin reactions in tuberculosis, 125.

Pregnancy, infections of, antistreptococ-
cus serum in, 826; general considera-
tion of, 827; normal serum in treatment of,
824; normal horse serum in treatment of,
823; pyelitis in, due to colon bacillus, 242; serodiagnosis of, 142; see also Serodiagnosis of pregnancy; vac-
cines in treatment of, 827.

Preservatives, use of, in antimeningitis
serum, 532, 533.

Primary anemias, classification of, 799;
definition of, 796.

Primary idiopathic anemia, serum and
blood treatment of, 808–810.

Proeschel, use of Ferrán method of treat-
ing rabies by, 732.

Prophylactic inoculation against Malta
fever, 661.

Prosperol, 326.

Prostate, involvement of, in gonococcal
septicemia, 578.

Prostatitis, complement-fixation test in,
140; metastatic lesions following, 578; vac-
cine treatment of, 617.

Protein content of cerebrospinal fluid,
516.

Protein disintegration in tissues, due

to action of bacteria, a cause of toxic
manifestations, 202.

Protein disintegration of bacillus in
body tissues, a cause of toxic manifes-
tations, 200.

Protein of tubercle bacillus, essential
constituent of, 328.

"Protein poison" of Vaughan, 16.

Proteins, foreign, intravenous injections

Proteolysis of bacterial protein in
body, cause of bacterial infection
(Vaughan's theory), 17.

Promethrin, absence of, in hemorrhagic
disease of the newborn, 796.

Protozoa in the normal mouth, 635.

"Pseudodysentery bacillus," 250.

Pseudo gonococcus, 557.

Puérperal infection, antistreptococcus
serum in treatment of, 826; general
consideration of, 827; vaccines in treat-
ment of, 827.

Pulmonary infarction, due to gonococcal
infection, 587.

Pulmonary tuberculosis, increasing doses
of tuberculin in treatment of, 342; tu-
bercle bacilli in blood of patients with,
312; see also Tuberculosis.

Purpura, arthritic, serum treatment of,
814; blood platelets and, 797; in gon-
occocemia, 588.

Purpura hemorrhagica, serum and blood
treatment of, 814.

Purpura rheumatica, 814.

Purpura simplex, 814.

Pus tubes, antistreptococcus serum for
post-operative treatment on removal of,
827.

Puscardin, method of, for attenuating virus
by heat, 728.

Pyelitis, complicating cerebrospinal men-
ingitis, 553.

Pyelitis due to bacillus coli, 239; acute,
course of, 240; acute septic, in typhoid
fever, 241; chronic, differential diagno-
sis of, 242; chronic ulcerative, in
typhoid fever, 241; chronic uncomplicat-
ed, continuous drainage treatment of,
246; complicated, treatment of, 244; in
pregnancy, vaccine therapy in, 245; in
typhoid fever, symptoms of, 241; pre-
disposing factors for, 239; treatment of, 244; uncomplicated, vaccine therapy for, 244.

Pneumonia due to bacillus coli, 242.

Phlebitis due to bacillus coli, 241; chronic bacillus coli, differential diagnosis of, 242.

Pyogenic meningitis, bacterial examination of cerebrospinal fluid in, 518.

Pyorrhea, arthritis and, 635; specific treatment of, 637.

Pyosalpinx, metastatic lesions following, 578.

Quarantine in epidemic meningitis, 564–567, 575.

Quinin, use of, in pneumococcal infections, 496.

Quinin hydrobromid, use of, in Graves' disease, 792.

Rabbit serum, method of obtaining, 803; use of, in pneumonia, 476.

Rabbits, inoculation of, for production of fixed virus, 720.

"Rabio tuberculat," 704, 707.

Rabies, animals affected by, 705, 706; bibliography on, 741, 742; case history in, 715; constitutional prophylactic treatment of, 718, 719; definition of, 701, 702; diagnosis of, 715–717; etiology of, 708–711; geographical distribution and prevalence of, 704–707; gross pathology of, 707; histologic pathology of, 707, 708; history of, 702, 704; ill effects of treatment of, 738–741; incubation period in, 712; local prophylactic treatment of, 718; mortality in, 725, 727, 738; Pasteur treatment of, 719–726; results of treatment of, 737, 738; seasonal prevalence of, 706, 707; serum-vaccine treatment of, by Roumanian method, 732, 733; symptoms of, 713–715; symptoms of, in the dog, 713, 714; symptoms of, in man, 714, 715.


Rabies, virus of, 708, 709; attenuation of, by carabolic acid, 729; attenuation of, by glycerin, 729; attenuation of, by heat, 728, 729; attenuation of, by gradual drying, 724–728; attenuation of, by rapid drying, 727, 728; filtrability of, 710; fixed, 719, 720; modification of, by dialysis, 729, 730; powdered, 727, 728; response of, to physical and chemical agents, 709, 710; street, 719, 720; unmodified fresh, 731; use of, with serum, 732, 733.

Rabinowitz, Lydia, on tubercle bacilli in the blood, 310.

Ransom, on absorption of tetanus toxin and antitoxin, 458; on direct antitoxin injections in treatment of tetanus, 460.

Rash, in acute ulcerous gingivitis, 637; in gonococcal septicemia, 591; in gonococemia, 588.

Battenskje antivenin, 747.

Reiss and Jungmann on convalescent scarlet fever serum therapy, 643.

Reiter's vaccine, 610.

Renal infarction, due to gonococcal infection, 587.

Renal insufficiency, due to gonococcal infection, 587.

Renal tuberculosis, post-operative treatment of, with tuberculin, 322; removal of kidney in, 322.

Reschd-Bey, acid-fast streptotheses isolated in leprosy cases by, 383.

Respiratory embarrassment following injection of antitenringitis serum, 530.

Respiratory organs, anaphylactic reaction in, 51.

Re, in gonococcal infections, 607; in Graves' disease, 792.

Revaccination in typhoid fever, 196.
RHEUMATIC FEVER, acute, vaccine therapy in, 175.
RHEUMATISM, acute articular, gonococcal arthritis resembling, 590; biologic characteristics in, 510; etiology of, 506; isolation of streptococci in, 507; microorganisms isolated in lesions of, 506; specific therapy of, 511; streptococci cause of, 506, 507.
RICHTER, experiments of, on anaphylaxis, 36; on transferable sensitization in anaphylaxis, 96.
Ringer's solution for treatment of dermatoses of pregnancy, 825.
RIVIERE AND MORLAND, on autotoxic and localized tuberculinosis, 342.
ROCKY MOUNTAIN SPOTTED FEVER, serum therapy in, 658.
RODAKEN, 733.
ROBERT ULCER, simulated recession of, 771-773.
Roentgenotherapy in Hodgkin's disease, 664.
Rogers, work of, on antistreptococcus serum, 650.
Rogers' and Beebe's serum, for Graves' disease, 794.
Rogers' treatment of cholera, with intravenous injections of hypertonic saline solution and administration of permanganate of potash, 291.
RÖMER, intracutaneous quantitative tuberculin test of, for tuberculin hypersensitiveness in animals, 348; on demonstration of antibodies in sera of immune animals, 335; on identical effects of tuberculin from human, bovine and avian tubercle bacilli, 329; on relation between tuberculin reaction and hypersensitive reaction following reinoculation, 337; on action of tuberculin, 328.
RÖMER AND VIERECK, on the influence of antitoxin in post-diphtheritic paralysis, 447.
Römer's polyvalent serum, use of, in pneumonia, 477, 478; in ulcer cornæ serpens, 502.
Rongy, on induction of labor by human serum, 824.
ROSACEA, vaccine therapy for, 633.
ROSE COLD, etiology of, 673.
ROSENNAU, on antianaphylaxis, 98.
ROSENNAU AND ANDERSON, experiments of, on anaphylaxis, 38; methods of sensitization of, in experimental anaphylaxis, 41; on dosage of sensitization in experimental anaphylaxis, 40; on influence of antitoxin in post-diphtheritic paralysis, 446; on specificity of sensitization in experimental anaphylaxis, 42.
ROSENBAUH, tuberculín of, 326.
ROSENOW, on alterations of biologic and cultural characteristics of pneumococcus by variation of media and environment, 204; on virulence of pneumococci, 12.
ROSENOW, results of, in vaccine treatment of Hodgkin's disease, 662.
ROSENOW AND HEETZEN, use of autolyzed vaccines by, in pneumonia, 491, 492.
Ross, on use of arsenic and mercury in scarlet fever, 647.
Best treatment of leprosy with "leprolin," 389.
Roth, on typhoid agglutination tests in tuberculosis, 211.
ROTHBERGER AND WINTERBURY, on functional cardiac disturbances in anaphylectic rabbit, 63.
ROTKY, experiments of, in anti-aggressin serum against cholera, 286.
ROUX AND BORREL, on antitoxin injection treatment of tetanus, 460.
ROUX AND YERSIN, on toxins of diphtheria, 434.
ROUX'S METHOD OF STANDARDIZATION OF DIPHTHERIA ANTITOXIN, 437.
ROVING, use of unaltered vaccines by, for cancer, 776, 777.
Buck's watery extract in treatment of tuberculosis, 317.
RUDOLPH, treatment of leprosy by, with nastin, 393.
RUMP, on convalescent scarlet fever serum therapy, 643.
RUSSO-JAPANESE WAR, typhoid losses of Russian army in, compared with losses from other causes, 188-189.
RUTHERFOORD'S treatment of leprosy with vaccine of Williams' culture, 392.
<table>
<thead>
<tr>
<th>Term</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sabouraud, experiments of, for acne bacillus,</td>
<td>624.</td>
</tr>
<tr>
<td>Sahli, on danger of rapid reactions in tuberculin treatment,</td>
<td>341.</td>
</tr>
<tr>
<td>Sakaguchi and Watabiki, on cutaneous tests with gonococcal toxins,</td>
<td>602.</td>
</tr>
<tr>
<td>Salimbene and Orticoni, on treatment of Asiatic cholera by, with serum enemata,</td>
<td>293.</td>
</tr>
<tr>
<td>Salimbene's treatment of cholera cases in St. Petersburg,</td>
<td>291.</td>
</tr>
<tr>
<td>Salivary glands, anaphylactic reaction in,</td>
<td>80.</td>
</tr>
<tr>
<td>Salol, use of, in serum sickness,</td>
<td>558.</td>
</tr>
<tr>
<td>Salt solution, prophylactic use of, in meningitis epidemics,</td>
<td>566.</td>
</tr>
<tr>
<td>Salvarsan, Herxheimer reaction following, 400; influence of, on Wassermann reaction in syphilis, 137; intraspinous injections of, 410; relative value of neo-salvarsan and, 409; use of, in blastomyocosis, 428; use of, in syphilis of central nervous system, 408; use of serum containing, in syphilis, 410-418; with complement-containing serum of animals, in treatment of leprosy, 383.</td>
<td></td>
</tr>
<tr>
<td>Salvarsanized serum for intraspinal treatment of tabes and paresis, 14.</td>
<td></td>
</tr>
<tr>
<td>Sandes, treatment of leprosy by, with suspension of killed leprosy bacilli, 391.</td>
<td></td>
</tr>
<tr>
<td>Sanitation in prophylaxis against typhoid, 197.</td>
<td></td>
</tr>
<tr>
<td>Sarcoma, Hodgkin's disease and, 663; use of unaltered vaccines in, 777.</td>
<td></td>
</tr>
<tr>
<td>Scarlet fever, antistreptococcus serum in, 645; arsenic in treatment of, 647; cause of, 642; convalescent scarlet fever serum for treatment of, 643; mercury in treatment of, 647; neo-salvarsan in treatment of, 647; secondary complications of, streptococcus cause of, 642; secondary infections of, vaccine treatment of, 646; specific remedies in, 642; value of antistreptococcus serum in, 646.</td>
<td></td>
</tr>
<tr>
<td>Scarlet fever, streptococcus vaccines as prophylactic measure in, 644; administration of, 644; preparation of, 644; results of, 644; statistics of, 644; value of, 644.</td>
<td></td>
</tr>
<tr>
<td>Scarlet fever serum, convalescent, for treatment of scarlet fever, 643; administration of, 643; negative effect of, on lesions of secondary scarlet fever infections, 644; preparation of, 643; results from, 643.</td>
<td></td>
</tr>
<tr>
<td>Schick's test of immunity to diphtheria, 441.</td>
<td></td>
</tr>
<tr>
<td>Shiga, discovery of dysentery bacillus by, 249; first preparation of dysentery immune serum by, 260; on bacteriolytic action of antisyphiftic serum, 259.</td>
<td></td>
</tr>
<tr>
<td>Shiga's polyvalent serum of dysentery, results of, 260.</td>
<td></td>
</tr>
<tr>
<td>Shiga-Kruze dysentery bacillus, 249.</td>
<td></td>
</tr>
<tr>
<td>Schöttz, on eradicating diphtheria bacilli from throat following attack, by use of staphylococcus inoculations, 449.</td>
<td></td>
</tr>
<tr>
<td>Schittenhelm, on anaphylactoid phenomena, 105.</td>
<td></td>
</tr>
<tr>
<td>Schnoller, on results of tuberculin treatment, 318.</td>
<td></td>
</tr>
<tr>
<td>Scholz, on convalescent scarlet fever serum therapy, 643.</td>
<td></td>
</tr>
<tr>
<td>Scholtz and Klingsmuller, on use of lepromata in treatment of leprosy, 390.</td>
<td></td>
</tr>
<tr>
<td>Schottmüller and Barfurth, on use of vaccine in gonococcal pelvic infections, 617.</td>
<td></td>
</tr>
<tr>
<td>Schultze, on anaphylactic reaction on smooth muscle of viscera, 73; on reactions following use of gonococcal vaccines, 602.</td>
<td></td>
</tr>
<tr>
<td>Schumacher, treatment of leprosy by, with natin, 383.</td>
<td></td>
</tr>
<tr>
<td>Schurupoff cholera serum, results with, 290.</td>
<td></td>
</tr>
<tr>
<td>Schwenk and Kutscher, on allergic reactions in gonococcal infections, 601; on vaccine treatment of gonococcal arthritis, 613.</td>
<td></td>
</tr>
<tr>
<td>Sclavo's serum, 655.</td>
<td></td>
</tr>
<tr>
<td>Scott, treatment of leprosy by, with natin, 384.</td>
<td></td>
</tr>
<tr>
<td>Secondary anemia, acute, following hemorrhage, transfusion of blood in, 813; classification of, 799; definition of, 798.</td>
<td></td>
</tr>
<tr>
<td>Secondary meningitis, 514.</td>
<td></td>
</tr>
<tr>
<td>Secondary syphilitic meningitis, 404, 405.</td>
<td></td>
</tr>
<tr>
<td>Sedatives, use of, in cerebrospinal meningitis, 548; in serum sickness, 558.</td>
<td></td>
</tr>
<tr>
<td>Sensitization in anaphylaxis, heterologous, 96; homologous, 96.</td>
<td></td>
</tr>
</tbody>
</table>
INDEX

Sensitized vaccines, 28; value of, 30.
Sensitized vaccines in lesions produced
by coil communis, 247.
Sensitized vaccines in typhoid immuniza-
tion of man, 30; action of, 30;
preparation of, 30; use of, in gonococ-
cal urethritis, 612.
Sensitized gonococcal vaccines, 609; re-
action after, 611.
Septic pneumonia, complicating cerebro-
spinal meningitis, 553.
Septicemia, due to fusiform bacilli, 637;
gonococcal, see Gonococcal septicemia.
Sera and vaccines compared, 567, 568.
Serodiagnosis of pregnancy, dialyzation
method, 143; obtaining serum in, 143;
preparation of placental albumin in,
143; technique of, 144; testing the
dialyzers in, 143; value of, 144.
Serological diagnosis of typhoid fever, 206.
Serological diagnosis of whooping-cough, 298.
Serological reactions: agglutinins and
precipitins, 602, 603; complement-fixa-
tion, 603-605; in gonococcal infections,
602; opsonins, 603.
Serra, isolation by, from leprosy cases of
anaerobic acid-fast organisms, 383.
Serum, antianthrax, 655; antigenoncoccal,
618, 619; antimeningitis, see Antimen-
ingitis serum; antipneumococcal, see
Antipneumococcus serum; antirabic,
734, 735, see also Antirabic serum; anti-
toxic, in treatment of hemorrhage,
805; antistaphylococcous, 654; antitu-
mor, cytolytic effects of, on tumor cells,
758, 759.
Serum, definition of, 3; Deutschmann's,
654; human, in induction of labor, 824;
in diagnosis of sporotrichosis, 423, 424;
normal, see Normal serum; normal, in
treatment of intoxications of preg-
nancy, 824; normal horse, in treatment
of intoxications of pregnancy, 825; ob-
taining of, for diagnosis, 117; separa-
tion of, in large quantities, 118; separa-
tion of, in small quantities, 117; rela-
tive avidity of, 755, 756; relative spe-
cificity of, 755, 756; Römer's polyval-
ent, for pneumonia, 477, 478; Selavo's,
655.
Serum, heated, Wassermann system with,
132.
Serum, immune, of dysentery, 257; pro-
duction of, 259.
Serum of animals, complement-contain-
ing, with salvarsan, in treatment of
leprosy, 388.
Serum agglutination test for typhoid
fever, 206; degrees of agglutinability
depending on media bacilli are grown
in, 208; duration of reaction in, 207;
method of procedure in, 206; persist-
ence of reaction an index to immunity
in, 208; precautions in, 207; reaction
in, 209; reaction in, compared with
that for allied bacteria, 209; with sus-
pension of dead bacilli, 208.
Serum disease, anaphylactic manifesta-
tions of, in man, 90; treatment of, 103.
Serum enemata in Asiatic cholera, 293.
Serum-fastness, contributing causes of,
12; definition of, 11.
Serum reactions, in diagnosis of glanders,
657.
Serum sickness, complicating cerebro-
spinal meningitis, 554-558; precautions
against, 588-589; prophylaxis against,
557; treatment of, 557, 558.
Serum therapy in anemia and hemor-
rhagic diseases, 802-805.
Serum therapy in anthrax, 655.
Serum therapy in Asiatic cholera, 290.
Serum therapy in cancer, clinical data on,
773-775; experimental data on, 751-
762; Vidal's technique of, 773, 775.
Serum therapy in cerebrospinal syphilis,
409-419.
Serum therapy in cerebrospinal meningi-
tis, effect of, on cerebrospinal fluid,
520, 521; technique of, 414-418; re-
sponse to, 561, 562.
Serum therapy of chronic arthritis, 176.
Serum therapy in dysentery, dosage in,
262; results of, 260.
Serum therapy in epidemic cerebrospinal
meningitis, 521-540.
Serum therapy in epididymitis, 619.
Serum therapy in general bacteremia
preceding and during course of menin-
gitis, 535-540.
Serum therapy in gonococcal infections,
606, 607, 618, 619.
Serum therapy in Graves' disease, 793,
794.
Serum therapy in hemorrhagic diseases, 822; dosage of serum in, 822; obtaining of blood in, 823; preparation of defibrinated blood for, 823; preparation of serum for, 822; selection of donor in, 821.

Serum therapy in hemorrhagic diseases of the newborn, 822.

Serum therapy in hemorrhagic diseases with fibrinogen insufficiency, 796.

Serum therapy in leprosy, 387.

Serum therapy in Malta fever, 661.

Serum therapy in micrococccus aureus infections, 654.

Serum therapy of myositis, 176.

Serum therapy in plague, 266.

Serum therapy in pneumococcus infections, 473–487; use of ethylhydrocuprein with, 497.

Serum therapy in pneumonia, according to types of organisms, 483; effects of, 484, 485; experimental studies on, 473–476; summary of, 486, 487; use of camphor with, 501.

Serum therapy in prothrombin insufficiency, 796.

Serum therapy in rabies, 734–741.

Serum therapy in Rocky Mountain spotted fever, 658.

Serum therapy in staphylococcus infections, 654.

Serum treatment of systemic diseases, 174.

Serum therapy in typhoid fever, 226; immune serum in, 226; normal human serum in, 226; serum of convalescents in, 227.

Serum therapy in ulcus cornæ serpens, 502.

Serum therapy of uterine bleeding, 823.

Serum therapy in whooping-cough, 308.

Serum-vaccine treatment of rabies, 575.

Sewall, researches of, in snake venom antisera, 747.

Shattuck and Whittemore, on cutaneous reactions following use of gonococcal vaccines, 602.

Sheep serum, in treatment of Graves’ disease, 793.

Sill, on vaccine treatment of whooping-cough, 299.

Simmons and Frankel, first to attempt typhoid immunity by means of inoculations, 178.

Sinus drainage, in hydrocephalic conditions, 544.

Sinuses, infections of, vaccine therapy in, 639.

Skin lesions in gonococcemia, 588.

Smiley, on treatment of acne cases with vaccine, 631.

Smith, Theobald, active immunity against diphtheria produced by, with toxin-antitoxin mixtures, 442; experiments of, on anaphylaxis, 38; experiments of, regarding relation of duration of immunity and proportion of toxin and antitoxin in immune sera, 9; on indications for and against use of vaccines, 27; on treatment of acne cases with vaccine, 631.

Snake venoms, action of hemagglutinins of, in blood system, 743; action of hemolysins, 746; action of hemorrhagin, 746; action of neurotoxins, 746; amount of injection of antisera for, 747; antisera for, 746; history of researches on, 743; toxic constituents of, 743.

Soap solution, effect of, on pneumococcus, 13.

Sobnheim and Seligman, on alteration of biologic and cultural characteristics of typhoid bacillus by variation of media and environment, 203.

Soda, bicarbonate of, use of, in serum sickness, 558.

Sodium chlorid, for prevention of anaphylactic reaction, 100.

Spanish-American War, typhoid cases in American army in, statistics, 180; typhoid losses of American army in, compared with losses from other causes, 188–189.

Specific chemo-serological therapy, 13.

Specific Therapy, in immunity, 2; principles of, 1.

Spermatozoa, effect of immune serum upon, 757, 758.

“Spheroidal and oval-celled infiltration,” 704.
INDEX

SPirochaeta pallida infection. See Syphilis.

SPirochaeta buccalis, 635.

SPirochaeta dentium, 635.

"Spirochetemia," 398.

Spirochetes, distribution of, in paresis, 403; infection of the mouth by, 635, 636.

Spleen, enlargement of, in gonococcal septicemia, 591; enlargement of, in gonococcomia, 588; enlargement of, in Malta fever, 659, 661; involvement of, in Graves' disease, 791.

Splenectomy, transfusion of blood and, 810.

Splenitic anemia, serum and blood treatment in, 810.

Spondylitis deformans, following gonorrhea, 590.

Sponges, in treatment of serum sickness, 558.

Sporotrichum, 421.

Sporotrichum beurmanni, morphology and methods of detection of, 422, 423; pathogenesis of, 423.

Sporotrichosis, 421–424; etiology of, 421, 422; immunity against, 423; organism of, 421–423; serum diagnosis of, 423, 424.

Sprays, nasal and throat, in prophylaxis of meningitis, 564, 566, 575; use of, following antimeningococcus vaccination, 575.

Sputum, virulence of, in rabies, 716.

Stain, for demonstration of Negri bodies, 717.

Stammmer, use of autolyzed vaccine by, in cancer, 781.

Staphylococci, cultures of, in acne vaccine, 626.

Staphylococci meningitis, 514.

Staphylococcus, in Hodgkin's disease, 662.

Staphylococcus infections, serum therapy in, 654; derrmic vaccine therapy in, 623; chronic, autogenous preparation of, 623.

Staphylococcus sprays for eradicating diphtheria bacilli from throat following diphtheria attack, 449.

"Staphylococcus urethrae," 609.

Staphylococcus vaccine in treatment of furunculosis, 631; administration of, 632; autogenous, 631; dosage of, 633; preparation of, 632.

"Starvation fever," 262.

Steinhardt and Poon, on rabies virus, 711.

Stenberg, discovery of pneumococcus by, 468.

Stenberg and Telkin, on vaccine treatment of gonorrheal infection of female genitalia, 832.

Sticker, George, on etiology of hay fever, 671.

Stone, on injection of healthy serum into typhoid patients as therapeutic measure, 226.

Stools, obtaining bacillus typhosus from, for bacteriological diagnosis of typhoid fever, 204.

Strabismus in babies, 714.

Strauss reaction, in diagnosis of glands, 657.

Street virus, 719, 720.

Streptococci, cause of rheumatism, 506, 507; cause of secondary complications of scarlet fever, 633; hemolytic, arthritis caused by, 507; in Hodgkin's disease, 663; in rheumatism, isolation of, 507; killed, value of, in streptococcus immunity, 645; killed by other means than heat, value of, in streptococcus immunity, 645; living cultures of, value of, on streptococcus immunity, 645.

Streptococci meningitis, 514.

Streptococcus infections, antistreptococcus serum for, 859; antistreptococcus serum as prophylactic agent in, 652; dosage of antistreptococcus serum in, 652; local, antistreptococcus serum in, 652; technique of administration of antistreptococcus serum in, 652; value of antistreptococcus serum in, 653.

Streptococcus opsonin, production of 649.

Streptococcus vaccines in prophylaxis of scarlet fever, 644; administration of, 644; preparation of, 644; results of, 644; statistics of, 644; value of, 644.

Streptococcus vaccines in treatment of leprosy, 395.

Streptococcus viridans, endocarditis caused by, 507.

Streptothrix, species of, in actinomycosis, 426, 427.
SYRINGOTHRIX LEPROIDES, leprosy, 365.
"Strongyloides," complement-fixation test in, 140.
"Stupor in gonococcal infections," 588.
"Subarachnoid space, syphilitic involvement of, 401, 402.
"Subcutaneous gonococcal reactions," 159.
"Subcutaneous injection of dead gonococci," in diagnosis of gonococcal arthritis, 580.
"Subcutaneous tuberculin test," 151.
"Summer bronchitis," 669.
"Summer catarrh," 667; etiology of, 673, 674.
"Summer diarrhea," effects of Flexner serum on, 261.
"Suppuration, in actinomycosis," 429; eye, in cerebrospinal meningitis, 550; middle ear, complicating cerebrospinal meningitis, 551; in the lungs in actinomycosis, 430.
"Surgical procedures in local infectious diseases," 28.
"Surgical tuberculosis," continuous small dosage of tuberculin in treatment of, 342, 343; value of tuberculin treatment in, 333.
"Sweating, profuse, in gonococcemia," 588.
"Syphiosis vulgaris, vaccine therapy for," 632.
"Syphilis, absence of general immunity to," 397; cerebrospinal, value of Wassermann test in, 134; cerebrospinal fluid in 404-407; complement-fixation reaction in, 127, see also Wassermann reaction; cutaneous lesions of, 398; diagnosis of, by means of cobra venom solutions, 745; early treatment of, 400, 401; hereditary, value of Wassermann test in, 134; infectiousness of blood in, 398; latent, value of Wassermann test in, 134; Noguchi human cell reaction for, 134; Noguchi's modification of Wassermann test for, 134; of the brain, see Syphilis of central nervous system; primary, value of Wassermann test in, 134; secondary, value of Wassermann test in, 134; tertiary, paresis and, 403; value of Wassermann test in, 134; principles of treatment of, 403, 404.
"Syphilis, Luetic reaction in, 160; diagnostic value of, 162; in papular form, 160; in pustular form, 160; in torpid form, 162; negative, 160; positive, 160; preparation of antigen in, 160; result of treatment in, 162; technique of, 160.
"Syphilis of central nervous system, cell count of cerebrospinal fluid in, 404, 405; globulin content of cerebrospinal fluid in, 405; in patients receiving intensive but incomplete treatment, 400; Lange gold reaction in, 407; local treatment in, 409-419; pathology of, 401, 402; references on, 419, 420; salvarsan in treatment of, 408, 409; secondary, 402; serum therapy in, 409-419; specific treatment of, general considerations of, 397-408; tertiary, 402; tertiary, cell count of cerebrospinal fluid in, 404; treatment of, summary of, 418, 419; Wassermann reaction in cerebrospinal fluid in, 405-407.
"Syphilis, Wassermann reaction in, 127, see also Wassermann reaction; influence of mercurial treatment on, 135; influence of salvarsan on, 137; influence of treatment on, 134.
"Systemic diseases, focal infections and," 165; of focal origin, 167; of focal origin, chronic, pathology of, 168; prophylaxis of, 171.
"Systemic disease, results of secondary foci of infection in, 170; relation of suspected focus to, 168; site of focus in, 165; treatment of, 171; treatment of, removal of focus in, 174; after management in, 174; bacteriological examination in, 174; treatment of, vaccines and serum in, 174.

"Tabes dorsalis, salvarsanized serum for intraspinal treatment of," 14; cell count
of cerebrospinal fluid in, 405; globulin content of cerebrospinal fluid in, 405; pathology of, 402; serum therapy in, 411; use of salvarsan in, 408; value of Wassermann test in, 134; Wassermann reaction in cerebrospinal fluid in, 407.

TEEBAN, 324.
TEDESAPIN, 326.
TEEBUTT, on bacteriological diagnosis of typhoid fever by blood cultures, 203.
TEETH, infection and, 635.
TEMPERATURE, anaphylactic reaction in, 86.
TERESHINSKAYA AND POPOWA, on vaccine treatment of gonorrheal infection of female genitalia, 734.
TETANUS, antitoxic treatment for, procedure in, 466; bacilli of, source of, 453; bacterial poisons of, 453; diagnosis of, 454; differentiation of, from rabies, 716; endotoxins of, 457; means by which wound infection occurs in, 454; muscles involved in, 457; paths by which toxin of, reaches central nervous system, 455; period between absorption of toxin of, and development of symptoms, 456; presence of toxin of, in blood, 457; preventive treatment of, 454; results of antitoxic treatment of, in animals, 462; results of antitoxic treatment of, in man, 464; toxin of, absorption of, from tissues, 456; union of toxin of, with gray matter of brain and spinal cord, 456.
TETANUS, SPECIFIC treatment of, 457, see also under Tetanus antitoxin; by direct injection of antitoxin into central nervous system, 460; by subcutaneous injection of antitoxin, 460.
TETANUS ANTITOXIN, absorption of, from tissues, 450; antitoxic unit in, 458; foreign antitoxic units in, 458; persistence of, in blood, 458; protective action of, 457; production of, for therapeutic purposes, 458; results of use of, for immunization, 461; technique of testing, 458.
THALMAN'S BEEF AGAR MEDIUM, 592.
THAYER AND BLUMER, gonococcus isolated from blood by, 583.
THROMBIN, in defibrinated blood, 805; in normal serum, 804.
THROMBIN, use of, in hemorrhagic disease of the newborn, 796; in morbus maculosus neonatorum, 813.
THROMBOSIS OF THE PROSTATE VEINS, in gonococcemia, 588.
THRUSH, organism of, 424; pathogenesis of, 424.
THYROID, in Graves' disease, 791.
THYROID, detoxicatory property of, 792, 793.
THYROID DISEASES, immune-ferment reaction in, 145.
THYROID EXTRACT in treatment of Graves' disease, 792, 793.
THYROID GLAND, relation of, to brain, 790; relation of, to Graves' disease, 789, 790; relation of, to sexual organs, 789.
THYROIDECTOMY, 793.
TISSUE SPECIFICITY, 753-756.
TIZZONI AND PANICHI, mode of preparation of antipneumococcal serum, 477.
TODD, on antitoxic action of dysentery immune serum, 257; on bacteriological diagnosis of typhoid fever by blood cultures, 203.
TODD AND ROSENTHAL, investigation of dysentery bacillus toxins by, 254.
TONGUE, involvement of, in Graves' disease, 791.
TONSILLECTOMY in focal infection in tonsils, 172.
TONSILLITOMY in focal infection in tonsils, 172.
TONSILS, focal infection in, tonsillectomy for, 172; focal infection in, tonsillotomy in, 172; involvement of, in Graves' disease, 791.
TOR I VARIETY OF DYSENTERY BACILLUS, effect of specific serum on, 261.
TOXIC SERUM, in treatment of Graves' disease, 793.
TOXIC SUBSTANCES, production of, 17.
TRACHSILER, experiments of, for acne bacillus, 625.
TRANSFUSION of blood, direct, contraindicated in hemophilia, 812; direct disadvantages of, 807; in acute anemia following hemorrhage, 811; in chronic secondary anemia following hemorrhages, 811; in connection with operations, 812; in diseases of the liver, 816, 817; in gastric and duodenal ulcer, 815, 816;
INDEX

views as to immunity principles of, 327.

Tuberculin, initial dose of, 346; cutaneous tuberculin tests for, 346, 347; in different varieties of, 349; intracutaneous tuberculin tests for, 347; tests for, by estimating patient's tolerance for tuberculin, and injecting smaller dose, 346; tests for, by selecting safe dose below reacting dose, and increasing, 349.

Tuberculin, preparation of, by various methods of extracting tubercule bacilli: Beranek's Tuberculin, 325, Koch's Tuberculin-residue or New Tuberculin, 324; Landmann's Tuberculol, 325, von Ruck's Watery Extract, 325; from culture media in which tubercule bacilli have grown: Deny's Bouillon Fitrate, 324, Jochmann's Albumoe-free Tuberculin, 324, Koch's Original or Old Tuberculin, 323; from tubercule bacilli themselves: Koch's Bacilli-emulsion, 324.

Tuberculin, selection of a preparation of, 323, 329; protein of tubercule bacilli essential constituent in, 328; source of tubercule bacilli negligible factor in, 329.

Tuberculin hypersensitiveness, 336; and resistance to tuberculous reinfection, 337; and severity of infection, 337; in relation to tuberculous disease, course of, 338; prognostic value of, 337, 338; value of, 338.

Tuberculin reaction, 351; aggravation of intoxication symptoms at beginning of injections in, illustrative case, 357; constitutional symptoms in, 352; focal reaction in, 358; intoxication symptoms in, 356; intracutaneous, 157; local changes in, 361; local reaction in, illustrative cases, 359; loss of weight in, 337; percutaneous, 337; pulse rate increase in, 355; temperature elevations in, due to external influences, illustrative case, 354; temperature elevations in, due to intercurrent infections, 355; temperature in, 352; temporary variations of temperature in, 355.

Tuberculin test, cutaneous, 155; clinical value of, 156; technique of, 155; general reactions in, 156; negative reac-
INDEX

for, 332; post-operative, in renal tuberculosis, 322; preparation of tuberculin dilutions in, 344; rapid increase of dosage in the small amounts in, 362; repeated courses of, illustrative case, 372; results obtained from, 310; results obtained from, in animal experimentation, 310; selection of patient for, 331; subcutaneous injections in, 345; subsequent doses of tuberculin and intervals in, 350; terminal in, 371; tuberculin reaction in, 351, see also tuberculin reaction; value of, in surgical tuberculosis, 333.

Tuberculin treatment, initial dose of tuberculin in, 346; cutaneous tuberculin tests for, 346, 347; intracutaneous tuberculin tests for, 347; tests for, by estimating patient's tolerance for tuberculin, and injecting smaller dose, 346; tests for, by selecting safe dose below reacting dose and increasing, 349.

Tuberculin treatment in pulmonary tuberculosis, action of tuberculin in, 331; clinical results in, 311; clinical statistics in, 314, 316; early failure of, due to indiscriminate application of, 311; early results in, 311; favorable results in, from small dosage, cautiously increased, 313; lack of satisfactory classification a difficulty in reckoning results from, 314; method of continuous minimal dosage in, 333; method of increasing dosage in, 339; results of, in patients with quiescent lesions, 314; physical signs as an index to improvement in, 315; small dosage advocated in, 313; spontaneous improvement and regression in, 314; standards of comparison for judging results in, 316; statistics as to duration of life following, 319; statistics as to tubercle bacilli in sputum following, 320; symptoms as an index to improvement in, 315; value of, 322.

Tuberculin treatment in surgical forms of tuberculosis, 321; leprosy, 394; in tuberculosis of bones and joints, results of, 322; of female genital tuberculosis, 836; of serum membrane, tuberculin treatment in, 322; of tuberculosis of
genito-urinary organs, results in, 322;
of tuberculous adenitis, results of, 322;
of tuberculous laryngitis, results in, 321;
of tuberculous lesions of eye, results of, 321;
of tuberculous meningitis, 322;
of tuberculous peritonitis, 322.

Tuberculocidin, 324.
Tuberculonastin, 326.
Tuberculo-serovaccine of Meyer-Boppel, 324.

Tuberculosis, actinomycosis and, 427;
autotoxic, 342; autotoxic, large increasing doses of tuberculin at short intervals in, 342;
choice of tuberculin preparation in treatment of, see under Tuberculin; complement-fixation in, 141;
cutaneous tuberculin test in, 154; diagnosis of, by means of cobra venom solutions, 746;
haemorrhage in, 814, 815; localized, 342; localized, small equal doses of tuberculin at long intervals in, 343; miostaganin reaction in, 146;
of bones and joints, results of tuberculin treatment in, 322; of eye, tuberculin treatment of, results in, 321;
of genito-urinary organs, results of tuberculin treatment in, 322;
of kidney, post-operative treatment of, with tuberculin, 322;
of serous membrane, tuberculin treatment in, 322; principles of immunity regarding tubercle bacilli in, 326;
pulmonary, increasing doses of tuberculin in treatment of, 342;
subcutaneous tuberculin test in, 151.

Tuberculosis, surgical, continuous small dosage of tuberculin in treatment of, 342, 343;
tuberculin treatment of, 321;
value of tuberculin treatment in, 333.

Tuberculous Adenitis, results of tuberculin treatment in, 322.

Tuberculous Animals, immunity of, to reinfection, 336; reaction of, to reinoculations of tubercle bacilli, 335.

Tuberculous Arthritis, gonococcal arthritis resembling, 590.

Tuberculous Hypersensitiveness, tuberculin treatment in, 338.

Tuberculous Iritis, gonococcal iritis and, 583.

Tuberculous Laryngitis, tuberculin treatment of, results in, 321.

Tuberculous Lesions of the Eye, tuberculin treatment of, results in, 321.

Tuberculous Meningitis, bacterial examination of cerebrospinal fluid in, 518;
tuberculin treatment in, 322.

Tuberculous Peritonitis, tuberculin treatment in, 322.

Tuberculous Serum, agglutination of typhoid bacillus by, 210.

Turban, on results covering tubercle bacilli in sputum following tuberculin treatment, 320;
on results of tuberculin treatment, 318.

Turekhub, treatment of leprosy by, 391.

Typhoid Bacilli, agglutination of, by tuberculous serum, 210; bacterial proteolysis of, in tissues, cause of typhoid manifestations, 200; biologic and cultural characteristics of, altered by variation of media and environment, 203; cultural characteristics of, 233; immunity produced by final digestion of toxic protein of, by body ferments, 202; in duodenal fluid in relapse, 204; killed, use of, in immunization against typhoid, 179; protein disintegration in tissues due to action of, a cause of toxic manifestations, 202; selective action of, on certain tissues, 201; susceptibility of certain tissues to, 200; virulence of, in presence of bacillus coli, 200.

Typhoid Cases in American army in Spanish-American War, statistics of, 180;
in British army in Boer War, statistics of, 180; statistics of inoculated and unprotected, 180.

Typhoid Carriers, acute, treatment of, 222; epidemics traced to, 221; finding of bacillus typhosus in blood of, 206; finding bacillus typhosus in urine of, 206; immunity of, 199, 219; immunization of attendants to prevent their becoming, 221; statistics of typhoid cases due to, 221; symptoms of, 219; treatment of, other than vaccine, 222; vaccine treatment in, 219, 222.

Typhoid Epidemics traced to typhoid carriers, 221.

Typhoid Fever, agglutination reaction in, 120; American losses by, in Spanish-American War compared with losses from other causes, 188-189; antityphoid
INDEX

vaccine for, in children, dosage of, 195; antityphoid vaccine for prophylaxis against, in service and civil life, 195; bacterial and serum therapy in, general considerations, 199; bacterial therapy of, 216; blood and serum treatment of hemorrhage in, 814; British losses from, in Boer War, 188-189; chart showing admission rates for, 193; chart showing death rates from, in United States, 194; chart showing non-effective rates for, 194; differentiation of, from gonococcal infection, 591; clinical, due to paratyphoid organisms, 211; cutaneous diagnostic reaction in, 210; duration of immunity conferred by antityphoid vaccine in, 196; epidemic, use of antityphoid vaccine as prophylactic measure in, 196; etiology of, 200, 201; German losses from, in Franco-German War, 188-189; gonococcal septicemia simulating, 588; immunity in, produced by final digestion of bacterial protein by body ferments, 202; kidney inflammations caused by colon bacillus in, 241; liberal dietary in, to relieve subsequent "starvation fever," 202; manifestations of, due to breaking up of bacterial protein in tissues, 200; manifestations of, due to breaking up of protein of tissue cells by bacteria, 202; ophthalmic diagnostic reaction in, 200; prophylactic vaccination for, see under Antityphoid vaccine; prophylaxis in, against epidemic, by antityphoid vaccination, 216; prophylaxis in, by means of vaccines, 178; see also under Antityphoid vaccine; prophylaxis in, by vaccines, history of, 178-182; prophylaxis in, general sanitation for, 190; relapse in, causes of, 205; revaccination in, 206; serological diagnosis of, 216, see also Serum agglutination test for typhoid fever; Russian losses by, in Russo-Japanese War, 188-189; serum therapy in, 226; specific and non-specific factors in, 32; tissues attacked in, 200; treatment of, by sensitized vaccines, 30; typhoid-ophthalmo reaction in, 158; vaccination for, in German Army, 182; vaccination for, in United States Army, results of, 188-192; vaccination for, with antityphoid vaccine in cases of those already infected with, 196; vaccine of Vincent used in France for immunisation, 186; vaccine treatment of complications of, 219; virulence of, and presence of secondary bacteria in body, 200.

Typhoid fever, bacteriological diagnosis of, 202; by blood cultures, 203; by clot-cultures on sterile bile, 206; by cultures of typhoid bacillus obtained from bile, 204; by examination of stools, 206; by examination of urine, 206.

Typhoid fever, serum agglutination test in, 206; degrees of agglutinability depending on media bacilli are grown in, 208; duration of reaction in, 207; method of procedure in, 206; persistence of reaction an index to immunity, 208; precautions in, 207; reaction in, 209; reaction in compared with that for allied bacteria, 209; with suspension of dead bacilli, 208.

Typhoid immunisation of Vincent, in France, 193.

Typhoid immunisation, sensitized vaccine for, 30; action of, 30; preparation of, 30.

Typhoid statistics in United States Army during recent years, 190-192.

Typhoid vaccination, 583; of British Army in Boer War, unfavorable results of, explained, 181.

Typhoid vaccines, clinical data concerning use of, in typhoid cases, 214; experimental data concerning use of, in people in health, 213.

Typhoid-opthalmo reaction, 158; antigen preparation in, 158; technique of, 158.

Uhlenhuth on specificity of sensitisation in experimental anaphylaxis, 42.

Uhlenhuth and Mulzer, studies of, on infectiousness of blood in syphilis, 398.

Ulcus gastrici and duodenal, transfusion of blood in, 817, 818.

Ulcerative endocarditis, gonococcal, 584.

Ulcus cornuæ serpens, 502; optochin in treatment of, 503; serum therapy in, 502; vaccine therapy in, 502.
Unaltered vaccines, in treatment of cancer, 775–779.
Unbulant fever. See also Malta fever.
Unna, experiments of, for acne bacillus, 624.
Urethane, for prevention of anaphylactic reaction, 100.
Urethra, inflammation of, due to colon bacillus, 238.
Urethral gonorrhea, spontaneous healing of, 599.
Urethritis, acute anterior, complement fixation test in, 140; bacillus coli cause of, 238; chronic gonorrheal, bacillus coli in, 243; chronic posterior, complement fixation test in, 140; gonococcal, vaccine treatment of, 611, 612; metastatic lesions following, 573.
Urinary antiseptics in treatment of acute typhoid carrier, 222.
Urinary diseases, treatment of, to prevent focal infection, 173.
Uriner, anaphylactic reaction in, 82; obtaining bacillus typhoecus from, for bacterial diagnosis of typhoid fever, 206.
Uretroplax, prophylactic use of, in meningitis epidemics, 566, 567, 575; use of, following meningococcus vaccination, 575; use of, in cerebrospinal meningitis, 548.
Uterine bleeding, serum in treatment of, 825.
Uterine cancer, antistreptococcus serum in post-operative treatment of, 829.
Urticaria, in serum sickness complicating cerebrospinal meningitis, 555.
Uveitis, gonococcal, 618.

Vaccination, in experimental tumors, 763–766; in plague as prophylactic measure, 266; prophylactic, in Asiatic cholera, 280; prophylactic, in Malta fever, 661; prophylactic, in pneumonia, 493, 494; prophylactic, in typhoid fever, 178. 568, see also Vaccine therapy and Vaccines; with antityphoid vaccine in case of those already infected, 196.

Vaccine, meningococcus, clinical reaction to, 571, 572; dosage of, 572; preparation of, 570, 572, 573, 575; prophylactic, use of, 569, 570, 575; use of, in arthritis complicating cerebrospinal meningitis, 552.

Vaccine therapy, in actinomycosis, 431; in alveolar abscess, 637; in blastomycosis, 428; unaltered, in treatment of cancer, 775–779; in cancer, clinical data on, 775–782; in cancer, experimental data on, 762–769; in chronic meningitis, 540; in diseases of unknown etiology, 640; in empyema, 493; in epididymitis, 613, 614, 616; in erysipelas, 637–639; in fusiform bacillus infections, 636; in glanders, 657; in infections of the sinuses, 639; in Malta fever, 661; in otitis media, 639; in pneumonia, 487–494; in pneumonia, results of, 489–491; in pyorrhea, 637; in rabies, indications for, 718, 719; in supplicative processes of the ear, 639; in ulcus cornae serpens, 502.

Vaccine therapy of acne, 628; in cases of comedones, 630; in cases of deep-seated nodules and abscesses, 629; in cases of superficial pustules, 629.

Vaccine therapy in acute rheumatic fever, 175.

Vaccine therapy of Asiatic cholera, 280.
Vaccine therapy for carbunculosis, 633.
Vaccine therapy of cystitis due to bacillus coli, 244.

Vaccine therapy for deforming arthritis, 175.

Vaccine therapy in dermatology, 623.
Vaccine therapy in dysentery, 262; results in, 263.
Vaccine therapy for eczema, 633.

Vaccine therapy of furunculosis, 631. See also under Staphylococcus vaccine.

Vaccine therapy in gonococcal infections, 606–608; gonococcal ophthalmia, 613; gonococcal vaccines, 611, 612; method of, 611; urethritis, 611, 612; vulvovaginitis, 612, 613.

Vaccine therapy of gonorrheal infections of female genitalia, 831; results of, 833.

Vaccine therapy in infections of the ear and sinuses, 639.

Vaccine therapy in infectious diseases, 19; clinical evidence for and against, 25; conditions favorable to, 19; dos-
INDEX

Vaccine therapy of leprosy, 389; non-specific, 394.
Vaccine therapy for myositis, 175.
Vaccine therapy of paratyphoid fever, 216.
Vaccine therapy in plague, 266.
Vaccines in therapy of puerperal infection, 829.
Vaccine therapy in pyleitis due to colon bacillus during pregnancy, 245.
Vaccine therapy of pyleitis, uncomplicated, due to bacillus coli, 244.
Vaccine therapy for rosacea, 633.
Vaccine therapy, sensitized, for typhoid fever, 30, 218.
Vaccine therapy for sycoosis vulgaris, 632.
Vaccine therapy in typhoid carrier, 219, 222.
Vaccine therapy of typhoid complications, 219.
Vaccine therapy of vulvovaginitis, 835.
Vaccine therapy of whooping-cough, 299; technique of administration of vaccine in, 301.
Vaccine therapy of wounds infected by bacillus coli, 246.
Vaccine for treatment of typhoid, preparation of, 225; results in, analysis of, 216; results in, discussion of, 218.
Vaccines, 488; and serums compared, 567, 568; antityphoid, see Antityphoid vaccine; as a means of reducing mortality in whooping-cough, 305; for acne, 626; for prophylaxis of whooping-cough, 305; in treatment of systemic diseases, 174; sensitized, 28; streptococcus, in prophylaxis of scarlet fever, 644; typhoid, clinical data concerning use of, in typhoid cases, 214; typhoid, experimental data concerning use of, in people in health, 113; autolyzed, in treatment of cancer, 779-781; autolyzed, in treatment of pneumonia, 491-492; commercial, 640; diagnostic use of, in gonococcal infections, 600-602; Doyen's, in treatment of cancer, 783; in treatment of heart complications in cerebrospinal meningitis, 551, 552; in treatment of Hodgkin's disease, 661-663; in treatment of mild chronic meningitis, 541; in treatment of relapse in cerebrospinal fever, 558; in treatment of severe chronic meningitis, 540; mixed, 639; purpose of, 567.
Vaccines, gonococcal, allergic reactions following use of, 600-602; commercial, preparations of, 610; dosage of, 638, 609; preparation of, 608, 609; sensitized, 609, 610; size and intervals of inoculations of, 610, 611.
Vaginal fistula, antistreptococcus serum for post-operative treatment of, 827.
Vaginitis, gonococcal, complement-fixation reaction in, 608; therapeutic use of lactic acid bacilli in, 833.
Vaillard and Dopter, on action of toxins of dysentery bacillus, 254.
Valvular involvement, gonococcal, 584.
Van de Velde, on allergic reactions in gonococcal infections, 601.
Van Gehuchten and Nelis, discoveries of, in rabies, 708.
Varnet and Clark, experiments of, on acne bacillus, 625.
Vaughan, experiments of, on proteolysis of bacterial protein, 17; method of separating in vitro bacterial protein poison freed in tissue cells, 201; on anaphylactoid phenomena, 105; on causes of anaphylactic shock, 141; on typhoid manifestations due to bacterial proteolysis under influence of tissue cells, 200, 201; theory of, on anaphylaxis, 107; theory of, on proteolysis of bacterial proteins in body, as cause of bacterial infection, 17.
Venomous serum, in treatment of leprosy, 389.
Ventricular puncture, dangers of, 544; in posterior basic meningitis, 542.
Vernon in treatment of cerebrospinal meningitis, 548.
Verteuil, treatment of leprosy by, with nartin, 393.
Venereal, complement-fixation test in, 140.
Vidal, use of serum therapy by, in cancer, 773-775.
INDEX

VINCENT, antityphoid vaccine of, preparation of, 186; work of, in France, with antityphoid vaccine, 193.
VINCENT AND BELLOT, diagnostic precipitin test of, for epidemic meningitis, 125.
VINCENT'S ANGINA, fusiform bacilli in, 636.
VIRCHOW, on mobilization of tubercle bacilli and a spread of the disease following tuberculin treatment, 311; on tuberculin treatment of pulmonary tuberculosis, 311.
VIRULENCE OF BACTERIA, contributing causes of, 12.
VIRUS, RABIES. See Rabies virus.
VIRUS UNIT, 735.
VOMITING, in rabies, 714; pernicious, of pregnancy, normal serum in treatment of, 825.
Von Reck's watery extract, preparation of, 325.
VULVOVAGINITIS, in children, complement-fixation test in, 140; vaccine treatment of, 612, 613, 833.

WARM Sponging, in treatment of cerebrospinal meningitis, 548.
WASSERMANN AND BRUCK, views of, as to immunity principles of tuberculin, 327.
WASSERMANN AND KOLLE, preparation of specific immune serum for cerebrospinal meningitis by, 521.
WASSERMANN, NEISSER AND BRUCK, on complement-fixation in syphilis, 127.
WASSERMANN AND STRONG, on immunity from inoculation of aggressive exudates in cholera investigations, 286.
WASSERMANN REACTION OF CEREBROSPINAL FLUID, 418; in syphilis of central nervous system, 405-407.
WASSERMANN REACTION IN SYPHILIS, 127; clinical value of, 134; complement-fixation with spirochetal antigen, 133; influence of mercurial treatment on, 135; influence of salvarsan on, 137; nature of, 138; Noguchi modification of, 130.
WASSERMANN REACTION IN SYPHILIS, technique of, 127; apparatus used in, 127; choice of method in, 130; reagents used in: amoebocytor, 130; antigen, 128; complement, 129; erythrocytes, 129; patient's serum, 127; standardization of reagents: amoebocytor titration, 130; determination of new antigen unit, 131; determination of syphilitic unit, 131; use of heated serum in, 132.

WASSERMANN TESTS IN DISEASES OTHER THAN SYPHILIS, 134.
WATABIKI AND SAKAGUCHI, cutaneous tests by, with gonococcal toxins, 602.
WATER, fear of, in rabies in man, 702, 714.
WATTERS, analysis of results in bacterial therapy of typhoid by, 216, 217; on use of prophylactic streptococcus vaccines in scarlet fever, 644.
WEAVER AND TUNNICLIFFE, on antistreptococcus serum in septic scarlet fever, 646; on treatment with antistreptococcus serum, 826.
WEBER AND DIETERLEIN, on identical effects of human and bovine tuberculin, 329.
WHICHHARDT, gramisol, hay fever serum of, 688.
WEIGHT'S LAW OF OVER-COMPENSATION, 7.
WEIL, on treatment of meningitis, 102; use of cobra venom solutions by, in diagnosis of syphilis, 745.
Weir Mitchell rest cure in Graves' disease, 792.
WEISSBACHER, on convalescent scarlet fever serum therapy, 641.
WELLS, on dosage of sensitisation in experimental anaphylaxis, 40.
WELLS AND OSBORNE, on specificity of sensitisation in experimental anaphylaxis, 42.
Wertheim, studies of, on immunity to gonorrhea, 599.
WHITE, quantitative cutaneous tuberculin tests of, for administration of tuberculin, 347.
WHITE AND GRAHAM, views of, on action of tuberculin, 328.
WHITEFIELD, experiments of, for acne bacillus, 625.
WHITEMORE AND CLEGG, treatment of leprosy by, with vaccines prepared from organism isolated by Clegg, 390.
WHITEMORE AND SHATTUCK, on reactions following use of gonococcal vaccines, 602.
Whole blood, intravenous use of, 307, 308.

Whooping-cough, bacillus of, history of, 296; discovery of, bacillus of, by Bordet and Gengou, 296; diagnosis of, 296; duration of, under various treatments, 304, 305; mortality in, 305; serodiagnosis of, 299; serum therapy in, 308; technique of administration of vaccine in treatment of, 301; vaccine as a means of reducing mortality in, 305; vaccine for prophylaxis in, 305; vaccine treatment of, 299.

Widal, on serum agglutination test for typhoid fever, 206.

Widal reaction, 119, 120; comparison between gross and microscopic tests in, 123; diagnostic value of, 123; macroscopic method, 121; microscopic reaction in, 121; typhoid culture in, 120.

Williams, on Negri bodies, 710, 711; treatment of leprosy by, with vaccine of streptothrix from leprosy lesions, 391.

Williams’ spread method for demonstrating Negri bodies, 716.

Wilson, theory of, on Graves’ disease, 790.

Wise and Minett, treatment of leprosy by, with naftin, 393.

Wolbach and Honer, on organisms cultivated from leprosy cases, 383.

Wolff-Eisner, conjunctival-tuberculin reaction of, 158; on organ specificity in sensitization in experimental anaphylaxis, 42; on protein content of tuberculin, 328; on tuberculin hyperreactivity, 337.

Wolves, rabies in, 705.

Wood, G. B., on hay fever, 669.

“Woody tongue,” 431.

Wooley, on treatment of leprosy with leprous nodules, 389.

Wright, experiments of, with vaccination on pneumonia, 493, 494; investigations of, on continuous minimal doses in tuberculin treatment, 333; observations of, on opsonins in localized infections, questioned, 21; on killed typhoid bacilli inoculations for typhoid immunizations, 179; on opsonic index in vaccine therapy of cutaneous diseases, 628; on small doses of vaccine for immunization, 20; on opsonic curves in infectious diseases, 20; on vaccine therapy in dermatological infections, 623; statistics of inoculated and unprotected typhoid cases in British Army in Boer War, 180; treatment of furunculosis by, with staphylococcus vaccine, 631; typhoid immunisation in British Army by, 179; work of, on bacterial vaccines, 488.

Wyman, Morrill, on etiology of hay fever, 669.

X-ray, in diagnosis of gonococcal arthritis, 590; in treatment of cancer, 784; in treatment of Graves’ disease, 793.

Yates and Bunting, experiments of, in Hodgkin’s disease, 662.

Yersin serum, 278.

Yellow fever, fibrinogen insufficiency in, 796.

Zinsser and Dwyer, experiments of, in cholera toxin, 286.

Zinsser and Hiss, treatment of pneumonia by, with leukocyte extract, 495.

Zsigmondy, findings of, on colloidal gold reaction, 3.