Infection, immunity and serum therapy : in relation to the infectious diseases which attack man; with considerations of the allied subjects of agglutination, precipitation, hemolysis, etc. / by H. T. Ricketts.

Contributors

Ricketts, Howard Taylor, 1871-1910.
Harvey Cushing/John Hay Whitney Medical Library

Publication/Creation

Chicago : American Medical Association Press, 1906.

Persistent URL

https://wellcomecollection.org/works/h35yn8tb

License and attribution

This material has been provided by This material has been provided by the Harvey Cushing/John Hay Whitney Medical Library at Yale University, through the Medical Heritage Library. The original may be consulted at the Harvey Cushing/John Hay Whitney Medical Library at Yale University. where the originals may be consulted.
This work has been identified as being free of known restrictions under copyright law, including all related and neighbouring rights and is being made available under the Creative Commons, Public Domain Mark.

You can copy, modify, distribute and perform the work, even for commercial purposes, without asking permission.

wellcome collection

Wellcome Collection
183 Euston Road
London NW1 2BE UK
T +44 (0)20 7611 8722
E library@wellcomecollection.org
https://wellcomecollection.org

15.5D
R

YALE
MEDICAL LIBRARY

HISTORICAL
LIBRARY
The Harvey Cushing Fund

ARGCAaf/

1st ed. 1906

(Preface dated 1906
© 1905)

Digitized by the Internet Archive
in 2012 with funding from
Open Knowledge Commons and Yale University, Cushing/Whitney Medical Library

http://archive.org/details/infectionimmunit1906rick2

INFECTION, IMMUNITY AND SERUM THERAPY

In RELATION *to the* INFECTIOUS DISEASES *which* ATTACK MAN; WITH CONSIDERATIONS *of* THE ALLIED SUBJECTS OF AGGLUTINATION, PRECIPITATION, HEMOLYSIS, ETC.

BY

H. T. RICKETTS, M.D.

Instructor in Pathology, University of Chicago

CHICAGO
AMERICAN MEDICAL ASSOCIATION PRESS
103 DEARBORN AVENUE
1906

Copyright, 1905.
by
American Medical Association.

Hist
RC111
R52
1906
(locked)

PREFACE.

Immunity, in its present state of development, with its manifold new terms and special methods of experimentation, is a subject which appears difficult to one who has not studied the newer literature assiduously and grown into a knowledge of the conditions through actual work in the laboratory. Much of the literature is technical in character and appears in journals not commonly found in the hands of the physician and student. Much of it also is comparatively recent, and its "essence" has not yet appeared in books which are in general use. The literature of immunity, moreover, grows so amazingly that the analysis even of current works is a task of no mean proportions.

At the same time, the subject is one of great interest and importance, and there exists a general wish, frequently expressed, to know more about the recent advances and the conditions which have operated against the success of serum therapy on a broader scale.

The editor of *The Journal of the American Medical Association,* appreciating the need which seemed to exist, requested the writer to prepare a series of articles on the subject of "Immunity,"

which should present the general principles and the important theories and facts, in as simple a manner as possible. These articles appeared from week to week during the year of 1905 in the journal mentioned, and after revision, and with such additions as would contribute to the completeness of the work, they are now collected, in larger type, in the present volume and under a more suitable title.

It was thought best to treat the subject broadly, to begin with the fundamental principles of infection and resistance and to introduce the reader to the more complex conceptions of the present time by taking him briefly over the main historical and developmental steps.

It will be obvious that the views of Ehrlich, concerning the production of antibodies, the nature of the reactions into which the latter enter, and the methods by which bacteria produce disease, have been utilized extensively. This course demands no justification, when it is appreciated that by no other means can one at the present time correlate a multitude of well-established facts which bear on the problems of immunity. Whatever may be the eventual fate of the side-chain theory—and certain phases of it carry the aspect of finality—we should appreciate as much as possible the extent to which it has shaped modern thought, and recognize

that it has won an imperishable place in the history of biologic progress.

It should also be understood that the utilization of the side-chain theory in no sense carries with it a negation of the importance of phagocytosis, a fact which is plainly set forth on pages 212-215. Without doubt the rôle of the phagocytes in recovery from a large group of infections is on a better and truer basis than it has ever been before, and for this condition the recent work on opsonins has been most significant.

In relation to the grouping of the infectious diseases adopted in Part Two, attention is called to the explanatory paragraph on page 235.

It will be noted that a complete bibliography of the subject of immunity has not been added, and this needs no explanation to one who is conscious of the massive proportions of the literature. A critical analysis of the entire literature, which would not have been in harmony with the endeavor to present the topics briefly and clearly, and which would have made detailed references essential, has not been attempted. The works cited in the bibliography are largely analytic in character and carry with them more or less complete references, through which those who are so interested may reach the original sources easily. This is true particularly of the handbook of Kolle and Wasser-

mann and of the volume by Metchnikoff. Almost the entire fourth volume of the former is devoted to the subject of immunity, the enormous literature of which is analyzed by different persons of international repute. The most recent literature on the various subjects is accessible through the *Index Medicus,* or the index prepared semi-annually by *The Journal of the American Medical Association.*

The index at the close of this volume serves as a glossary of terms, the explanations of which may be found on the pages referred to.

H. T. RICKETTS.

CHICAGO, February, 1906.

CONTENTS.

PART ONE—GENERAL.

CHAPTER I.

Parasitism, Infectiousness, Contagiousness..... 1- 6

CHAPTER II.

Infectious Etiology..... 7- 16

CHAPTER III.

General Considerations..... 17- 23

CHAPTER IV.

History and Development..... 24- 34

CHAPTER V.

Natural Immunity:

A. Protection Afforded by the Body Surfaces 35- 41
B. The Protective Nature of Inflammation.. 41- 46
C. The Antibacterial and Antitoxic Nature of Natural Immunity..... 46- 55

CHAPTER VI.

Acquired Immunity..... 56- 64

CHAPTER VII.

Toxins and Antitoxins..... 65- 77

CHAPTER VIII.

The "Structure" of Toxins and Antitoxins and the Nature of the Toxin-Antitoxin Reaction... 78- 91

CHAPTER IX.

The Phenomena of Agglutination..... 92-104

CHAPTER X.

The Nature of the Substances Concerned in Agglutination 105-118

CHAPTER XI.

Precipitins 119-129

CHAPTER XII.

A. General Properties of Bactericidal Serums...130-141
B. Amboceptors and Complements.....141-161

CHAPTER XIII.

Cytotoxins 162-175

CHAPTER XIV.

Phagocytosis 176-194

CHAPTER XV.

The Side-Chain Theory of Ehrlich and Its Relation to the Theory of Phagocytosis.....195-217

CHAPTER XVI.

Principles of Serum Therapy.....218-234

PART TWO—SPECIAL.

GROUP I.—ACQUIRED IMMUNITY IS CHIEFLY ANTITOXIC.

A.—Bacterial Diseases:

Diphtheria 235-244
Tetanus 244-256
Botulism 256-259
Bacillus Pyocyaneus.....259-262
Other Soluble Bacterial Toxins..... 262

B.—Intoxication by Soluble Plant Toxins:

Hay Fever.....262-264
Other Plant Toxins..... 264

C.—Intoxication by Soluble Animal Toxins:

Poisoning by Snake Bites.....264-268
Other Zoötoxins..... 268

GROUP II.—ACQUIRED IMMUNITY IS CHIEFLY ANTIBACTERIAL.

Typhoid Fever.....269-284
Paratyphoid Fever.....284-288
Acute Epidemic Dysentery.....288-294

Meat Poisoning by Bacillus Enteritidis.....294-298
Bacillus Coli Communis.....298-304
Cholera 304-315
Plague 315-327
Anthrax 327-333
Malta Fever.....333-335

GROUP III.—ACUTE INFECTIONS IN WHICH LASTING IMMUNITY IS NOT ESTABLISHED.

Pneumococcus Infections—Pneumonia 336-349
Streptococci 349-370
Staphylococci 370-383
Micrococcus Catarrhalis 383-384
Gonorrhea and Other Infections with the Gonococcus 384-388
Epidemic Cerebrospinal Meningitis.....388-393
Influenza 393-399
Soft Chancre 399-401
Bacillus of Friedländer and Other Members of the Capsule-Forming Group.....401-402
Relapsing Fever 403-406

GROUP IV.—CHRONIC INFECTIONS IN WHICH LASTING IMMUNITY IS NOT ESTABLISHED.

Tuberculosis 407-445
Leprosy 445-452
Glanders 452-458
Rhinoscleroma 458
Actinomycosis 458-462
Madura Foot..... 462
Infections by Streptothrix, Cladothrix and Leptothrix 463
Oidiomycosis 463-467

GROUP V.—DISEASES OF PROTOZOÖN ETIOLOGY.

Malaria 468-483
Trypanosomiasis 483-497
"Spotted Fever" of the Rocky Mountain States..497-498
Texas Fever as an Example of Pyroplasmosis...499-500
Amebic Dysentery.....500-504

Sarcosporidia 504
Balantidium Coli 505
Cercomonas Intestinalis..... 506
Trichomonas 507
Coccidiosis 508

GROUP VI.—DISEASES OF DOUBTFUL OR UNKNOWN ETIOLOGY.

Hydrophobia 510-521
Syphilis 522-529
Yellow Fever.....529-538
Typhus Fever..... 538
Dengue Fever.....539-541
Acute Articular Rheumatism..... 541
Smallpox and Vaccinia.....541-555
Chickenpox (Varicella) 555
Scarlet Fever 556-559
Measles 559-562
German Measles (Rötheln)..... 562
Whooping Cough.....562-566
Mumps 566

Appendix 567-571
Bibliography 572
Index 573-599
Corrections 600

PART ONE—GENERAL.

CHAPTER I.

PARASITISM, INFECTIOUSNESS, CONTAGIOUSNESS.

Parasitism. Parasitism is the condition in which a plant, or an animal being, lives on or within another living organism. A true parasite always derives its sustenance from the tissues of its host.

Some parasites may live on a host without causing appreciable damage, that is, they are non-pathogenic parasites. In this case they may derive their nutrition from some of the excreted non-living products of the host, living as pure saprophytes,[1] or the amount of nutritious substance which they obtain from the host may be so little that the health of the latter is not impaired.

There is another large class of parasites, however, which under proper conditions cause severe diseases in the host. Many pathogenic microbes live in and on the skin without doing harm, but if certain ones reach the deeper tissues, they may institute pathologic processes (e. g., staphylococci). Any organism which is able to cause pathologic tissue changes or to set up abnormal symptoms is classed as a pathogenic parasite. The abnormal processes which they set up are our infectious diseases.

1. A saprophyte is defined as a vegetable organism which lives on dead organic matter. An organism which is habitually saprophytic may become pathogenic under the proper conditions (bacillus of malignant edema). And, on the other hand, a pathogenic parasite lives a saprophytic life, when it grows in our artificial culture media.

Infectious Disease.

An infectious disease is one which is caused by living organisms which in some way have been introduced into the tissues of the body. Accordingly the word has reference to the nature of the cause of the disease. It is from the Latin *inficire,* meaning to place in or into.

Infestation and Infection.

Where living organisms exist on a body surface, as the skin or intestinal tract, the surface is said to be infested; the skin, for example, is infested with pediculi. One may also say that the intestinal tract is infested with tape worms, but here the distinction between infestation and infection is not to be drawn so sharply; surely when the larvæ penetrate the intestinal wall and reach the circulation or distant organs we must speak of infection. But even the adult tenia as it exists in the intestines may cause erosions of the mucous membrane or may perhaps burrow a slight distance into the wall, a condition which approximates the action of the larvæ in passing through the wall; accordingly at some point the distinction between infestation and infection becomes an arbitrary one.

Contagiousness and Infectiousness.

Confusion sometimes arises in using the words infectious and contagious. A contagious disease is one which may be transmitted from one individual to another by direct or indirect contact; the word has reference to the manner of transmission of an infection. Non-infectious diseases are never contagious. Contagiousness is well illustrated in those disease in which the transmission takes place through the air, as seems to be the case in smallpox and scarlet fever. Here there may be a contagious zone of atmosphere surrounding the patient, in which the virus is present, and

by which the agent reaches the lungs of one within the zone. Contagiousness is even more striking when it takes place through the medium of some inanimate substance, such as clothing or toys, which were previously within the contagious zone or in direct contact with the patient. Such substances, fomites, were formerly thought to be of great importance in the extension of yellow fever; a theory which has been entirely exploded by the proof that the disease is transmitted through the bites of infected mosquitoes.

Contact Infection.

Typhoid fever is not highly contagious. There probably is no infected zone of atmosphere about the patient in the sense that there is about a scarlet fever patient. Yet it frequently happens that the nurse contracts the disease while caring for her patient. It is likely that she has in some way transferred the bacteria from the patient's sputum, urine, feces or skin to her lips or hands so that eventually they found their way into the intestines. At the same time it is not improbable that the point of entrance for the germs, i. e., the infection atrium, may at times be the lungs, the bacteria having been inhaled. However, typhoid fever is usually a "water borne" disease, seldom "air borne."

Infection Atrium.

We are to look on malaria as a strictly non-contagious disease, although no doubt is possible as to its infectiousness. No amount of personal contact with the patient will transmit it to another. To accomplish transmission the parasite must be actually injected into an individual, an event which happens exclusively, it is believed, through the bite of a mosquito which has previously fed on the blood of a malarial patient.

Tetanus is another noted example of an infectious disease which is not acquired by contact with one suffering from it.

Pathogenesis. The various parasites have different ways of injuring the body. The itch mite burrows into and beneath the epidermis, causing mechanical irritation as well as the inflammatory reaction produced by its toxic excretions. The loss of blood caused by some intestinal parasites, and the hemorrhage which follows from the wounds, often lead to grave anemias and the general changes which are caused by such anemias. This is especially the case in the duodenal infection with uncinaria duodenalis (uncinariasis).

A certain variety of *Filaria sanguinis hominis* produces disorders by mechanically blocking the lymph channels.

The plasmodium of malaria causes anemia by the destruction of blood cells.

The febril disturbances of infectious diseases are certainly due to toxic influences.

Mechanical and Toxic Injuries. It is generally said that bacteria produce disturbances in both a mechanical and a toxic way. However, the more we learn about bacterial infections the more important do the toxic phenomena become. It is doubtful if any pathogenic bacterium is entirely devoid of toxic powers.

It is probable that the bacterial emboli which are sometimes found in capillaries and small arteries cause disturbances through the shutting off of so much circulation, but still greater damage may result from the action of toxins which are formed by the microbes making up the emboli.

In lobar pneumonia we have a good example of a mechanical disturbance of importance. The

alveoli become filled with a fibrinous and purulent exudate which makes a large area of pulmonary tissue unavailable for respiration. Yet, even here, the mechanical disturbance has arisen only as a result of the previous toxic action of the pneumococci on the capillary walls and the alveolar epithelium, permitting the escape of the blood and serum.

Effects of Toxins.

The toxins of bacteria often show a remarkable selective action on one organ or another. Some destroy the red blood cells to a great degree (staphylococcus and streptococcus); other toxins have a special affinity for the nervous tissue (tetanus); some attack particularly the endothelium of the vessels, causing many minute hemorrhages. Frequently bacterial toxins cause areas of necrosis in the lymphoid and parenchymatous organs, and the granular degenerations of the latter, in acute infectious diseases, are well known. The albumin and casts which appear in the urine in many acute febrile diseases are the result of toxic action on the epithelium and endothelium of the kidneys. It is said that in anthrax all the glycogen may disappear from the liver; toxins may disturb the functions of various organs to similar degree.

Some chronic infections are characterized by the development of new connective tissue and vessels; this is seen especially in tuberculosis, syphilis and actinomycosis. The import of the new connective tissue depends on its location. A large amount of it may form in pre-existing connective tissues with no consequent harm; but even a small scar, gumma or tubercle in the brain may cause serious results.

Infectious Substances.

It has been stated above that infectious diseases are caused by living pathogenic organisms. Investigations have shown, however, that the toxic

products of some organisms can be prepared and separated from the organisms themselves by filtration, and that such microbe-free toxins when injected into animals may cause the same symptoms that are produced by the bacteria themselves (tetanus and diphtheria). Accordingly, for the sake of convenience, these toxins also may be considered among the infectious agents, even though separated from their corresponding bacteria. The various infectious agents, including these toxins, find their proper places in the following classification, which, for the most part, is that of von Behring:

I. Living (i. e., pathogenic parasites).
   A. Macroparasites (e. g., intestinal worms, pediculi).
   B. Microparasites.
      1. Bacteria (fission fungi: each cell divides into two in proliferating).
      2. Fungi of more complex organization (e. g., aspergillum, oidia).
      3. Protozoa (e. g., *plasmodium malariæ, ameba coli*).

II. Non-living (i. e., toxins).
   A. Animal toxins (e. g., snake venom).
   B. Vegetable toxins.
      1. Non-bacterial (e. g., abrin, from the jequirity bean; ricin, from the castor oil bean; these are strong red corpuscle poisons, chiefly of experimental interest).
      2. Bacterial.
         a. Soluble bacterial toxins (diphtheria and tetanus).
         b. Intracellular bacterial toxins, which are not secreted by the cells in a soluble form.

CHAPTER II.

INFECTIOUS ETIOLOGY.

The microbic cause of a disease must be in hand before a logical attempt can be made to prepare a specific immune serum. It is not in all cases necessary that the organism be cultivated artificially, however; the conditions in rinderpest may be cited in which the body fluids of a diseased animal, known to contain the infectious agent, are used for immunization, although the microbe itself can not as yet be cultivated or recognized.

Koch's Laws.

There are so many possibilities of error, and so many errors have actually been made in regard to infectious etiology, that certain requirements in the way of proof are now habitually demanded before a particular organism can be accepted as the cause of a disease. These requirements are most frequently expressed in the form of Koch's laws, which may be stated as follows 1. The suspected organism must be found constantly in the proper tissues of an animal suffering from the disease, or which has died from it. 2. The organism must be cultivated artificially in a pure state. 3. It must be possible to reproduce the disease in a suitable animal by inoculation with the pure culture. 4. The organism must again be cultivated in a pure state from the tissues of the experiment animal.

Agglutination Test.

Since these laws were formulated another procedure has been developed which may give valuable evidence as to etiology. This pertains to the agglutination test, or, as we speak of it in connection with typhoid fever, the Gruber-Widal reaction.

This principle, that in the acquiring of immunity to a microbic infection the serum of an individual gains in agglutinating power for the micro-organism, has been found to hold true in many infections. Consequently, if one has in hand the specific micro-organism for a disease, he would expect the serum of a patient sick of this disease to have a stronger agglutinating power for this micro-organism than for others which were accidentally present; and this power would also be greater than that possessed by the serum of one who had not had this particular disease. In spite of some possibilities of error the agglutination test has been of distinct value in the recognition of the specific micro-organisms in certain diseases, as in the case of the germ of epidemic dysentery (Shiga).

Obstacles to Koch's Laws.

All Koch's laws have not been complied with in certain cases, because of various difficulties which have been encountered. First, the pathogenic protozoa can not be cultivated on artificial media (we must except the success of Novy and McNeal with certain trypanosomas, and of Strong with the ameba coli under symbiotic conditions); second, certain bacteria which may be found constantly in a given disease have not been cultivated artificially (spirillum of recurrent fever); third, there are a few diseases which are peculiar to man and accordingly can not be reproduced in experiment animals (leprosy, influenza, scarlet fever, measles, etc.); fourth, some infectious agents are pathogenic for experiment animals, but do not reproduce in them a clinical or anatomic condition identical with that found in the original animal (typhoid).

Furthermore, failure to comply with all the re-

quirements enumerated does not, in some cases, disqualify the organism as the causal factor. If an organism is found constantly in characteristic sites in a given disease and not in other infections, and if at the same time other microbes are not present or are present inconstantly or through accident, there could be little or no hesitation in accepting this organism as the cause of the disease, even if it were impossible to cultivate it or to transfer the disease to animals. The typhoid bacillus has been cultivated from characteristic foci (stools, blood, spleen, urine, rose spots) in such a large number of cases, and the bactericidal and agglutinating powers of the patient's serum against this organism are so distinctive, that compliance with the third law, though desirable, is not now essential. The conditions are similar in reference to cholera and the cholera vibrio.

The conditions are so unique in some diseases that, although all Koch's laws have not been met literally, certain equivalents have been met. To illustrate, we may consider an anopheles mosquito which has become infected with the plasmodium of malaria by biting a malarial patient, as a culture medium; and the transferring of the infection to another patient by the bite of this mosquito as the inoculation experiment which is desired.

Specific Infectious Diseases.

The term "specific infectious disease" has come to have a very special meaning. It is applied to a disease having characteristic clinical and anatomic phenomena, which can be caused only by one particular micro-organism. Among the diseases which come within the limits of this brief definition, the following may be enumerated (the micro-organism which is the cause of each disease being also given):

Diphtheria	*Bacillus diphtheriæ*
Tetanus	*Bacillus tetani*
Typhoid fever	*Bacillus typhosus*
Cholera	*Vibrio choleræ*
Anthrax	*Bacillus anthracis*
Tuberculosis	*Bacillus tuberculosis*
Leprosy	*Bacillus lepræ*
Plague	*Bacillus pestis*
Dysentery (bacillary)	*Bacillus dysenteriæ*
Influenza	*Bacillus influenzæ*
Glanders (farcy)	*Bacillus mallei*
Chancroid	*Bacillus chancri mollis* (Ducrey)
Recurrent fever	*Spirillum obermeieri*
Gonorrhea	*Micrococcus gonorrheæ*
Epidemic cerebrospinal meningitis	*Diplococcus intracellularis meningitidis* (of Weichselbaum)
Actinomycosis	*Actinomyces bovis et hominis*
Blastomycosis	*Blastomycetes* and *Oidia*
Malaria	*Plasmodium malariæ*

A large number of animal diseases have their specific microbes, as do certain other human diseases which hardly concern us as to the subject in hand.

Unknown Etiology.

In addition to the diseases mentioned, there are several, of unknown etiology, which from analogy we must recognize as specific. Scarlet fever, measles, German measles, chickenpox, smallpox, yellow fever, typhus fever, hydrophobia and syphilis, are undoubtedly due to micro-organisms. Mallory recently found in the skin of four scarlet

fever patients a protozoön-like body which he believes to be the cause of the disease, although he admits that much desired proof has not been obtained. The *Diplococcus scarlatinæ* of Class, which a few years ago acquired some notoriety, has not been able to stand as the cause of scarlet fever in the face of rigid investigation.

Parker, Beyer and Pothier have announced the discovery of a protozoön (?) *Myxococcidium stegomyiæ,* in the mosquitoes which infect man with yellow fever; but Carroll maintains that this organism has no relation to the production of the disease. The *Bacillus icteroides,* which Sanarelli found in a rather high percentage of cases, is now generally considered as a contaminating organism.

It is possible that smallpox and vaccinia will be eliminated from the diseases of unknown causation, owing to the evidence of protozoön etiology that Councilman and his colaborers have obtained; however, for the present, the question may be considered *sub judice* in view of the fact that the forms described bear a close resemblance to certain well-known types of cell degeneration.

The following animal diseases, of unknown etiology, may also be mentioned in this connection: Foot and mouth disease, peripneumonia, bovine pest, sheep-pox (clavelee), chicken-typhus or chicken-pest and epithelioma contagiosum of fowls.

Obstacles to Discovery of Microbes.

The following appear to be the chief reasons for the failure to discover the organisms of these diseases: 1. Inability to cultivate the microbe. 2. Mixed, or symbiotic infections. It is conceivable that in some case the combined action of two micro-organisms may be necessary to cause the

disease. The non-toxic products of the two may synthesize to form a toxic substance (Hektoen.) 3. Unstained ability of the microbe. 4. Ultramicroscopic size. The organism of the peripneumonia of cattle was cultivated by Nocard and Roux by growing it in a closed collodion sac which was placed in the peritoneal cavity of suitable animals. It is so small that its form can not be made out, and growth is recognized only by clouding of the culture medium, and the increased virulence of the latter for animals.

Filterability of Viruses.

Some valuable information has been obtained by observing whether the infectious agents are so small that they will pass through dense filters of porcelain or infusorial earth. It has been found that the viruses of foot and mouth disease, peripneumonia, rinderpest, sheep-pox, chicken-typhus, horse sickness, epithelioma contagiosum of fowls, and yellow fever are filterable, i. e., they pass through the filter. This is determined by injecting the filtered culture medium or serum into susceptible animals. The viruses of smallpox and vaccinia are said to be non-filterable. Success in filtering the hydrophobia virus has recently been claimed by Remlinger. Inasmuch as scarlet fever, measles, chicken-pox, typhus fever and syphilis* can not be produced in animals, the filterability of their viruses is not at present susceptible of demonstration.

Non-Specific Infectious Processes.

There is a marked tendency in many diseases, typhoid, cholera, malaria, etc., for characteristic organs or groups of organs to be involved in some particular manner. These are features which

---

* Recently syphilis has been inoculated successfully into anthropoid apes. (Metchnikoff and Roux.)

stamp them as specific diseases. On the other hand, a large number of micro-organisms cause no well-defined clinical and anatomic disease, but, depending on various accidents, cause an inflammation now in one organ, now in another.

Suppuration is not a specific disease, because the pyogenic power is common to a large number of microbes. A diphtheritic or pseudo-diphtheritic process in the mouth and throat may be caused by the diphtheria bacillus, streptococcus, staphylococcus, oidium or yeasts; bronchitis may be caused by the influenza, tubercle, plague and typhoid bacilli, and by the infecting agents of the acute exanthemata, etc.; pulmonitis by the pneumococcus, streptococcus, tubercle, plague, Friedlander and influenza bacilli, oidium, actinomyces, etc.; meningitis by the tubercle and influenza bacilli, streptococcus, staphylococcus, pneumococcus, gonococcus, diplococcus of epidemic meningitis, the syphilis virus, etc.; arthritis by the streptococcus, staphylococcus, tubercle bacillus, gonococcus, the virus of rheumatic fever, etc.; endocarditis by the streptococcus, staphylococcus, gonococcus, pneumococcus, tubercle bacillus, etc., and septicemia by a whole host of organisms aside from those mentioned as causing specific diseases.

Within certain limits, however, there is often a degree of specificity in the processes produced by some of the organisms mentioned, which sometimes allows of clinical and anatomic differentiation. The infiltrating and rapidly extending invasion of the subcutaneous and connective tissues caused by the streptococcus can often be distinguished clinically from the slower, more circumscribed process of the staphylococcus. The condi-

tions induced by the *Bacillus aerogenes capsulatus*, the bacillus of malignant edema, are, in turn, different from those of the streptococcus and staphylococcus. The pneumococcus commonly causes the consolidation of rather extensive areas of the lung, whereas the streptococcus and the bacillus of Friedlander are more often found in the lobular consolidations. The membranous inflammation of diphtheria may in favorable cases be distinguished from that of the pyogenic organisms without bacteriologic aids; in this possibility, however, there lies no justification for neglect of the bacteriologic examination.

Mixed Infections.

The coexistence of two or more micro-organisms in a morbid condition is of frequent occurrence, and some of the most interesting and important phenomena of infectious diseases are referable to mixed, secondary or superimposed infections.

Two exogenous infections may attack an individual at the same time. Measles and scarlet fever and diphtheria and scarlet fever have been known to coexist. Pneumococcus pneumonia and typhoid fever, chancre and soft chancre with pus cocci, syphilis and gonorrhea, diphtheria with streptococci, tetanus with gangrene-producing organisms, are common observations. One organism may intensify the virulence of another. Diphtheria accompanied by streptococcus infection seems to be more virulent than diphtheria alone. It is also believed that the presence of aërobic organisms (those which demand oxygen for their development) in a wound infected with the tetanus bacillus or the bacillus of malignant edema (anaërobic organisms), may increase the virulence of these infections. Streptococci are probably important

organisms in scarlet fever, for they are present in unusual numbers in the throat lesions and are often found in fatal cases in all the organs, yet it is believed that they constitute only a mixed or secondary infection superimposed on that of the scarlatina virus. The conditions are somewhat similar in smallpox, the pustules of which invariably contain streptococci, staphylococci, or both. In both scarlatina and smallpox these secondary infections may be responsible for many fatalities.

Pneumococcus pneumonia occurring during the course of, or during convalescence from the eruptive fevers, diphtheria, typhoid fever or erysipelas; a streptococcus septicemia developing during typhoid (giving rise to an irregular temperature curve), streptococcus infection of tubercular cavities, and the development of acute tuberculosis during measles—these are important examples of secondary infections.

We should naturally expect that the presence of a severe secondary infection might embarrass attempts at serum therapy. Experience on this point is practically limited to diphtheria, and there is no lack of evidence to show that the disease when complicated by severe streptococcus infection often can not be controlled by antitoxin treatment.

Infectious Intoxications.

It may be emphasized that there are certain diseases in which a wide dissemination of the bacteria is not necessary for the production of morbid phenomena; where, in fact, they may be entirely localized (diphtheria, tetanus), and the symptoms are produced by virulent toxins which are readily dissolved in the body fluids and carried to important organs. More especially are those microbes

placed in this class which produce their specific disease-producing toxins in a soluble form when grown in our artificial culture media (bouillon). The bacilli of diphtheria, tetanus, botulism and the pyocyaneus bacillus belong distinctively to this group.

Snake venom and arachnolysin (spider poison) are the best known examples of soluble animal toxins. As will appear later, the serums of all animals probably contain a variety of toxins which are injurious to one or more species of animals.

CHAPTER III.

GENERAL CONSIDERATIONS.

Definition.

By immunity we understand that condition in which an individual or a species of animals exhibits unusual or complete resistance to an infection for which other individuals or other species show a greater or less degree of susceptibility. Immune is from the Latin *immunis,* which originally applied to one who was exempt from a public service, exempt from tribute, or free. Although the word retained this civil meaning for centuries, and still retains it in certain connections, it also had, even in ancient times, a limited application to the protection which an individual might possess against poisons. It is seen, for example, in descriptions of a tribe inhabiting Northern Africa, the Psylli, who possessed a natural immunity to the bites of poisonous snakes. Although we may be certain from this and other references that a condition of immunity was recognized in very ancient times, the present significance of the term has developed largely from a better understanding of the nature of infectious diseases and of the conditions upon which the resistance of the body depends.

Diseases Concerned In Immunity.

As the definition suggests, we do not think of immunity to such processes as Bright's disease, arteriosclerosis or the metabolic diseases, but only to those which we have learned to recognize as infectious.

Immunity has no necessary relationship to the degree of contagiousness of an infectious disease,

although some of the most striking and certainly the most common examples of immunity are seen in relation to such infections (as scarlet fever and smallpox). Tetanus, on the other hand, which is absolutely non-contagious, can likewise give rise to a high degree of immunity.

Acquired Immunity.

No medical fact is more widely known among intelligent people than that an attack of certain of our infectious diseases brings about some kind of change in the patient's tissues which protects him, or renders him immune, against further attacks of the same disease. Inasmuch as he was previously susceptible, the new property is an acquired one, and he is now said to possess an acquired immunity against this infection.

Natural Immunity.

It is also well known that many diseases which attack man can not be inoculated into animals, and biologists are familiar with many examples of immunity which are confined to particular species. The lower animals apparently can not be infected with scarlet fever or measles, nor man with chicken cholera. The negro is less susceptible than the white man to yellow fever. The resistance which these examples illustrate has occurred naturally, not through having the disease; it is a natural immunity.

Family Susceptibility and Immunity.

Natural immunity is, for the most part, an inherited condition; this certainly is the case where a whole class of animals is involved. Similarly, the susceptibility which is peculiar to a species must be hereditary. It is often said of some diseases that they run in families; e. g., carcinoma, gout, insanity. This appears to be just as true of some infectious diseases, the most noteworthy example of which is probably tuberculosis. In con-

trast to this inherited susceptibility is an inherited immunity, which may also run in families. It is not so easy to adduce examples of this. We are in the habit of thinking of the individual who can resist all infections as representing a standard. He, however, is above the average in resistance, and the average is our proper standard for estimating the resistance of a species or race of animals. It is undoubtedly true that some families possess an unusual resistance to tuberculosis. Furthermore, experimental work with animals has proved that, within limits, an immunity to certain infections (e. g., tetanus) acquired by a female may be transmitted to her offspring. Even in a given family, however, there are often marked variations in susceptibility and resistance. One child may contract scarlet fever, while his brother, living under exactly the same conditions, may escape it. There is, moreover, much reason to believe that the same individual varies greatly in his resistance at different times and under different conditions. Hence, the personal equation, as represented by individual resistance or individual susceptibility, is of no small consequence.

The facts mentioned were familiar long before anything was known regarding the principles on which they depend. Subsequent to the discovery of some of these principles (to be considered later), it became convenient and necessary to recognize other special types of immunity, although any type which can be conceived must still find a place under either natural or acquired immunity.

Antibacterial and Antitoxic Immunity.

Although such diseases as typhoid and cholera are accompanied by pronounced toxic symptoms, the poisonous substances seem to be integrally as-

sociated with the bacterial protoplasm and not secreted in a soluble or diffusible form by the living cell; they are spoken of as intracellular toxins or endotoxins. Observations point to the belief that the endotoxins are liberated only after the bacteria are killed and dissolved. When one, through infection, has acquired immunity to typhoid or cholera, his fresh serum is able to kill the respective bacterium, but apparently is not able to neutralize its toxic substance. Hence, on the basis of the nature of the serum, immunity to such diseases is spoken of as antibacterial rather than antitoxic. On the other hand, the symptoms which are so characteristic of tetanus are produced not by contact of the bacteria with the nervous system, but rather through the specific soluble toxin which is secreted by the bacilli in the wound where they reside. This poison, or toxin, is carried from the wound to the nervous system through the lymphatic or blood circulation, the bacterium itself not being transported. Therefore, although tetanus is a bacterial disease, it is at the same time and in a peculiar sense a toxic disease.

The serum of an animal which has acquired immunity to diphtheria or tetanus neutralizes the corresponding soluble toxin, but does not necessarily injure the micro-organism itself. That is to say, the immunity is antitoxic. Experience has shown that this distinction between antibacterial and antitoxic immunity is an important one, and the differentiation is very sharp in some instances. In many examples of natural immunity, the resistance can not be attributed so specifically to antibacterial or antitoxic serum properties. This is referred to later.

Active Immunity.

Immunity which results from an infection depends on a specific reaction on the part of the tissue cells in response to the chemical injury produced by the bacteria or their toxins. The indication of the occurrence of such a reaction lies, first, in the recovery of the patient, and, second, in the new antitoxic or antibacterial power which may be demonstrated in the serum. In view of the active part played by the body in establishing this new resistance, the condition is referred to as an active immunity. In the preparation of various antitoxic and antibacterial serums for commercial purposes, a condition of active immunity is deliberately produced in the animals (the horse, for example) by the injection of the toxins or of the bacteria.

Passive Immunity.

Contrariwise, the resistance which is established in an individual through the injection of an immune serum (such as diphtheria antitoxin) is a passive immunity, since it depends on the introduction of ready-made immunizing substances rather than on their production through an active process on the part of the one injected. Active and passive immunity, then, are varieties of acquired immunity. Depending on the disease which caused the immunity, or on the character of the serum injected, they may be either antibacterial or antitoxic.

Relative and Absolute Immunity.

Any one of the types mentioned may be either relative or absolute; synonyms are partial and complete. If absolute, infection is impossible. If only relative, different conditions may be made to prevail which would render infection possible; for example, a large number of bacteria will often cause an infection where a smaller number fails

to do so. There may also be a temporary decrease in one's resistance through overwork, hunger or exposure. Immunity is usually relative.

Classification of Types.

By proper combinations of the terms which have been enumerated, one may describe somewhat accurately the different forms of immunity. Thus, a child which has received a prophylactic injection of diphtheria antitoxin is in a state of acquired passive antitoxic immunity to diphtheria. If immunity to typhoid has developed as a result of the disease, the condition is that of an acquired active antibacterial immunity, etc. Accordingly, although the terms may be somewhat confusing, it is seen that they are in no sense contradictory.

The following classification of immunity is convenient, although in giving it, we must recognize that it probably does not include all types of immunity. At this point the single example may be cited, that the chicken is very resistant to tetanus toxin, although its serum contains no antitoxin; hence in this instance we could hardly speak of antitoxic immunity to tetanus, but rather of non-susceptibility to the toxin.

Immunity.	Natural.	Antibacterial or Antitoxic.
	The inherited immunity of species and varieties of animals.	
	Inherited family or individual immunity.	
	Acquired.	
	Active.	
	Passive.	

Drug Habituation.

In many of these conditions proper biologic experiments will demonstrate the presence of the antibacterial or antitoxic substance in the serum of the animal which possesses the immunity. The technic of such experiments will be illustrated later.

There is another kind of acquired resistance which properly may be mentioned here. It is a

well-known fact that one may gradually accustom himself to enormous doses of morphin, arsenic, alcohol and some other drugs. *A priori,* it would seem that this condition of resistance should be analogous to that which is present in antitoxic immunity. But, on the contrary, the serum of a morphin or alcohol habitué has no power of neutralizing the effects of morphin or alcohol. The conditions on which this resistance depends are not understood.

CHAPTER IV.

HISTORY AND DEVELOPMENT.

Early Times and Practices.

The conception of the nature of immunity which was current at one period or another of history had some relationship to the conception of the etiology of diseases at those times. It will be remembered that at one time diseases were supposed to be imposed by an angry deity, and to avert them various mysticisms were resorted to, such as the utterance of incantations and the wearing of talismans. On the other hand, a more logical attempt to explain the natural immunity of the Psylli against snake poison was made by Pliny, who suggested that it might be due to their habit of drinking water from wells in which poisonous snakes dwelt. This is not unlike our present conception of active immunization.

Von Behring quotes literature to show that among some primitive races of to-day, artificial immunization is carried on; a Mozambique tribe is said to inoculate against snake poison by rubbing into a small cutaneous incision a paste which contains venom. Probably non-fatal quantities are introduced in this way, resulting in the formation of venom antitoxin, a method comparable to that used in the production of diphtheria antitoxin.

At a very early period the possibility of habituation to poisonous drugs was recognized. We learn that Mithridates by taking gradually increasing doses of poisons established in himself resistance

of this sort. It is stated also that he fed ducks with poisons and then proposed to use their blood as an antidote (serumtherapy). The importance of antidotes in the minds of the ancients may be appreciated from the fact that epidemic diseases, such as plague, cholera and smallpox, were at one time considered as due to unknown poisons, which might be comparable in nature to some known poisons, as aconite. Mercury for syphilis, quinin for malaria, and salicylic acid for rheumatism would certainly have fallen into the category of antidotes, and mercury may have been so considered.

A historic illustration of the treatment of disease on a supposed etiologic basis is found in a theory which was prevalent in the seventeenth century, according to which diseases were either acid or basic in character, and hence should be treated, the one with an alkali, the other with an acid. Sylvius considered plague to be of acid nature and administered alkalies, while Etmüller took the opposite view.

Manifestly, rational treatment and prophylaxis of the infectious diseases could not be undertaken until their etiology was correctly understood. Yet here, as so often happens in medicine, empiricism preceded rationalism. For example, protective inoculation did not become a principle until the time of Pasteur, yet it had been practiced against smallpox for centuries, and the method put on its present basis by Jenner long before there was any idea as to the principles involved in the protection.

Micro-organisms.

The belief that invisible "animalcules" are able to cause morbid processes in man is a very old

one. A passage from Varro (116-27 B. C.) reads as follows: "There are swampy places in which grow animals never so small which may not be recognized by the naked eye, and which gain access to the body through the air and bring about severe diseases."

The Microscope.

The discovery and use of the compound microscope in the seventeenth century disclosed the reality of the minute living forms which had been suspected so often. Kircher, with his first crude microscope (1646), examined the tissues of various diseases, and was the author of many theories as to their etiology. It is now believed that the magnification of Kircher's microscope was so small that many of the "worms" which he saw were really larger fungus cells and in some instances the as yet undiscovered blood and pus cells.

Leeuwenhoek (1632-1723), a Dutch naturalist, with his compound microscope magnifying 1,000 diameters, described accurately many microscopic forms, but made no application of his discoveries to medical problems; nevertheless, such application was not wanting, and the succeeding century and a half saw such voluminous descriptions of microbes, so many contradictory theories and statements concerning their relationship to infections, that the "infinitesimally small" fell into disfavor in many quarters as the cause of diseases. The attractions and reasonableness of the theory, however, were such that it continued to gain exponents, and in the early part of the nineteenth century reached a degree of definiteness. In 1855, the great French physician, Brétonneau, affirmed that a specific germ was the cause of every con-

tagious disease: "An epidemic disease can originate and extend only through the agency of the germ producing it." Yet at this time no infection had been definitely proved to be of microbic origin.

Anthrax.

In 1850 Rayer and Devaine made an observation, which might have fallen into the oblivion of many preceding ones had it not been confirmed by later investigators. They found "small filiform bodies" in the blood of sheep which had died of anthrax, and were naturally inclined to believe that these forms caused the disease. Other stu- the study of anthrax, with the result that the the study of anthrax, with the result that the small rods of Devaine were scientifically proved to be its cause.

Fermentations.

Two great minds dominated medical research at this time—Pasteur and Koch. Pasteur, in his early career as a chemist, had had his attention called to the processes of fermentation. He recurred to this subject at a time when the theory of the spontaneous generation of small living forms was widely discussed, and in 1857-1861 proved beyond any possibility of doubt that lactic acid, alcoholic and butyric acid fermentations were due to the action of minute living cells; and, furthermore, that each particular kind of fermentation had its own peculiar microbe as the cause. This was an example of what we term to-day microbic specificity. Pasteur then applied what he had learned about fermentations to the study of the diseases of wines and beers. He found their causes, and devised a preventive measure, which consisted merely in the destruction of the germs by heating the wine to a suitable temperature be-

Microbic Specificity.

fore it was stored. At the instance of the French government, he then studied certain diseases of silk worms. His success in discovering their causes and prevention must always remain for us one of the landmarks of the world's progress. It was during the latter investigations that he took up the study of anthrax. The specific microbe having been discovered, and the methods of transmission of the malady having been made clear through investigations by both Pasteur and Koch, Pasteur turned his attention to methods of prevention and, if possible, of cure.

Vaccination. Pasteur pondered the question of smallpox vaccination. He came to believe that vaccinia was smallpox, the virus of which had been attenuated by its passage through the cow, and that consequently when man was vaccinated he was inoculated with a benign form of the disease. Might not this be an example of a law which would be general in its application? The protective inoculation (active immunization) against the pleuropneumonia of cattle which had long been practiced gave encouragement to this hope. Some work by Toussaint was important in the answer to this question. It was evident that a weakening or attenuation of the bacteria or virus must first be obtained before it could be safely injected into animals for the purpose of producing immunity, for if the unaltered virus were injected the virulent infection would result. Accordingly, Tous-

Anthrax. saint heated the blood of a sheep which had died of anthrax, to a temperature of 55° C. for ten minutes, then injected it into a number of sheep. Some of the animals died of anthrax, while others suffered only a mild attack from which they re-

covered; the latter were found to be immune to a subsequent inoculation with virulent blood. Inasmuch, however, as some of Toussaint's animals had died of anthrax, Pasteur concluded that there was some grave error in technic. He considered that Toussaint's method probably killed or attenuated the fully-developed bacilli, but did not injure the spores of the parasite (Koch had previously shown the existence of anthrax spores). After much experimentation, Pasteur hit on the plan of growing the bacillus at a temperature of 42° C., obtaining in this way a culture of the fully developed organism which had a low virulence, but which did not form the dangerous spores. When sheep were inoculated with the proper amount of this culture, which became known as anthrax vaccine, they had a mild attack of the disease, which rendered them immune to virulent inoculations.

Hydrophobia.

With the possibility of protective inoculation with a known virus actually demonstrated, similar procedures were tried with other animal diseases of known bacterial etiology, with the result that successful vaccines against chicken cholera and swine plague were developed. Somewhat later, having failed in their attempts to discover the microbes of plague and cholera, Pasteur and his co-workers turned to the study of hydrophobia. All efforts to cultivate the virus from the spinal cord of rabid dogs, which surely is its seat in the affected animal, failed. The unique idea then occurred to consider the infected spinal cord as a fully developed culture of the virus. It remained to subject such a culture to the proper attenuating conditions for the purpose of weaken-

ing or actually destroying its virulence in order to make it fit for protective injections. This was accomplished by drying the cords in a closed vessel over a hygroscopic substance (solid potassium hydroxid), the final virulence of the cord depending on the length of time it had been subjected to the drying process. The technic of the protective injections, the success of which is household knowledge, will be the subject of later consideration.

Two Important Principles.

Of primary importance, during this period, was the work of Koch on the specific bacteria of tuberculosis, cholera, typhoid and the pyogenic diseases; and not least his improved methods of obtaining pure cultures through the use of solid media (gelatin) on plates. Through his work and that of Pasteur two great principles had been set in motion; the microbic specificity of infectious diseases, and protective inoculation in its generalized form, through the use of attenuated virus.

Theories of the Cause of Immunity.

The scientific mind turned at once to the inquiry, What changes in an animal body are responsible for the immunity which is acquired as the result of protective inoculations? Also, upon what properties of the tissues or body fluids does the natural immunity of an animal depend, and does the susceptibility of one species depend on the absence of those properties which characterize the natural immunity of another species? Pasteur had observed that if he grew the microbe of chicken cholera in a liquid medium for some time, then removed the bacteria by filtration, the fluid became unfit for the further growth of the organism on subsequent reinoculation. That is, the nutrient material had been used up; and he sug-

Exhaustion Theory.

gested that this is the case in the body of an animal. Having undergone the infection, suitable nutrient material for the microbe is used up, and recovery ensues. The prolonged absence of the proper nutritious substances would account for the more or less permanent nature of the acquired immunity. This conception, the exhaustion theory, at one time shared by Koch and Klebs, is still represented in an altered form by Baumgarten, who speaks of an unfavorable culture medium as representing the condition of the immune body, which, of course, is broadly true.

Noxious Retention Theory.

Chauveau was the author of another historic theory of acquired immunity (the noxious retention theory), which maintained that during the course of a disease the bacteria produce substances in the presence of which they can not develop further; consequently recovery takes place, and the continued presence of these noxious substances renders another attack of the disease impossible. Although it is true that bacteria do not grow well in their own metabolic products, theories of immunity on this and similar bases are not in accord with the fact that immunity may be of great duration, and that it may be conferred by the injection of the killed bacteria, or, in some cases, of their non-living soluble products.

Phagocytosis.

Metchnikoff may be credited with having first offered a plausible explanation of natural resistance, founded on observation. As a zoologist he had studied the subject of intracellular digestion in the lower animals, and it was while working on this problem that he observed the fate of a yeast fungus (*Monospora*), which caused epidemics among the daphnia, small, transparent animals

with which he was working. Near the alimentary tract, which was the infection atrium, some large mesoblastic cells, which are perhaps analogous to the white blood cells, were seen to ingest the parasites and dissolve them. If this took place to a sufficient extent the animals recovered; if, however, the infecting organisms were too numerous or the reaction on the part of the animal insufficient, the body became overwhelmed with parasites and death resulted. Since that time Metchnikoff has evolved his well-known theory of phagocytosis as the essential factor in both natural and acquired immunity, a theory which Pasteur, in his later years, looked on with favor. We may speak of this as the cellular theory of immunity; a theory which has had to undergo important modications in order to bring it into accord with new facts.

Investigation of the Properties of Serums.

Considering that natural or acquired immunity must exist because of certain qualities of the body cells, or of the body fluids, or possibly of both, investigators began to make analyses of the tissues; and of all the analyses, that which we may term the biologic has been the most fruitful. In this case biologic analysis means the detection of reactions which may occur when bacteria or their products are placed on contact with tissue cells or fluids, either in the living animal or in test-glass experiments. The chief of these are the determination of the ability of the serum of an animal to kill bacteria or to neutralize bacterial toxins. These important investigations, still in their infancy, were inaugurated by the findings of Fodor, Nuttall, Nissen, v. Behring and Buchner, which showed that fresh defibrinated blood,

Bactericidal Power.

and the blood serum of various animals, were able to kill bacteria in the reagent glass. In contrast to the action of ordinary antiseptics, this power is often selective, killing one variety of bacterium and leaving another unharmed. This was of enormous importance, as it seemed to identify the factor on which natural antibacterial immunity depends. Then followed the discovery of Nissen and v. Behring (*Vibrio metchnikovi*), and of Bouchard (*B. pyocyaneus*), that if an animal is systematically injected, i. e., immunized, with a micro-organism, the power of its serum to kill the bacterium used in the immunization is greatly increased; from which it would seem that acquired immunity depends on the increase of powers which are normally present to a certain degree. These observations have to do with the bactericidal power of serum.

Toxins and Antitoxins.

Further progress was made through the discovery that the tetanus bacillus (Brieger and Fränkel) and the diphtheria bacillus (Roux and Yersin) secrete each a powerful, specific, soluble toxin, which may be separated from the bacteria by filtration. Immunization with these bacterium-free toxins was undertaken (Behring and Kitasato, 1890) with the familiar result of the production of the specific antitoxins. Other investigations in this direction soon showed the independence of the antibacterial and the antitoxic properties of serums.

With these facts in hand, the vigor with which investigations have been pushed may be readily imagined. The hope naturally prevailed that physicians might become the masters of all infectious diseases, through the possession of specific anti-

bacterial and antitoxic serums. But failures, with which we are only too familiar, met the attempts to produce adequate antiserums for many diseases. Nevertheless these failures, through stimulation to closer study, have resulted in the accumulation of much additional knowledge concerning the pathogenic properties of different bacteria, the nature of the immune serums and the various protective factors of the body. Ehrlich has evolved a new theory of immunity from facts which were discovered in his laboratory, the "side-chain" theory, which it is the purpose to utilize in the interpretation of many reactions which will come up for consideration.

CHAPTER V.

NATURAL IMMUNITY.

A. PROTECTION AFFORDED BY THE BODY SURFACES.

The Skin.

Virulent organisms (e. g., staphylococci and streptococci) exist normally on the skin or between the superficial horny cells, some exceptional circumstance being necessary, e. g., wounds, to enable them to penetrate deeper and to cause disease. It is evident, then, that the physiologic shedding of the superficial horny cells and their continual reformation at a deeper level is a process calculated to rid the surface of the body of many micro-organisms.

The question whether micro-organisms can ever penetrate the unbroken skin has been much discussed. Although experiments have shown that traumatism is not absolutely necessary, clinical experience indicates that these so-called cryptogenetic infections are not of ready occurrence. When they do occur, the infection atrium is probably one of the glandular orifices.

Cutaneous Orifices.

The sweat glands with their ducts, and the hair follicles with their appended sebaceous glands, are vulnerable points in the defense which the cutaneous surface represents. Although they are protected somewhat by the flow of their excretions, especially in warm weather, and although the entrance of germs is made more difficult by the contraction of the skin and consequent narrowing of the orifices in cold weather, yet various incidents may lead to the introduction and retention of

virulent micro-organisms in these structures. When this occurs there is little difficulty in the way of their producing necrosis of the epithelium, invading the surrounding tissue and causing a pustule, boil, carbuncle, cellulitis, or even a generalized infection. The secretion of the sebaceous glands appears to be not germicidal. On the other hand, the acid nature and certain salts found in perspiration render this fluid antagonistic to the development and virulence of certain micro-organisms.

The serous exudate, and the crust which forms subsequent to an abrasion, antagonize infection. The serum itself contains germicidal substances, while the crust mechanically prevents microbic invasion.

Soluble poisons such as aconite and bacterial toxins are not absorbed through the unbroken skin.

Subcutaneous Connective Tissue.

Even after germs penetrate the epidermis, the subcutaneous connective tissue is often an obstacle to their further extension. The subcutaneous injection of some micro-organisms (e. g., cholera) is tolerated better by animals than one given into the abdominal cavity or blood vessels. We are also familiar with the benign course of lupus compared with visceral tuberculosis; the same is true of cutaneous and visceral glanders. This resistance is explainable, at least in part, by the rapidity with which new connective tissue forms in the subcutaneous tissue, offering a mechanical limitation to the infection, and by the rich lymph supply which makes possible the rapid accumulation of bactericidal lymph and of phagocytic cells. On the other hand, it must be mentioned that in some diseases the subcutaneous tissue offers no

perceptible resistance to bacterial invasion (plague), and that toxins may be more virulent when introduced into this tissue than when injected into the abdominal cavity or the general circulation (tetanus).

Mucous Membranes.

The moist condition of mucous membranes has been found to favor the multiplication of many microbes, although mucus itself is said to attenuate the virulence of some micro-organisms, as the pneumococcus; mucus, however, is not actively germicidal. A layer of mucus, on the other hand, is a mechanical protection, and its constant excretion is a means of steadily removing bacteria from mucous surfaces.

Conjunctiva.

The conjunctiva is protected against infection by the mechanical interference of the eyebrows, eyelashes, eyelids, irrigation of the conjunctival surface by tears which carry germs through the lachrymal duct into the nasal cavity, the ability of the conjunctival epithelium to repair itself rapidly, and the mild germicidal action of the salts which are present in the tears. These protective agencies, however, are often surmounted by micro-organisms, such as the pneumococcus, staphylococcus and the influenza bacillus. Many soluble poisons, aconite, diphtheria toxin and the toxin of hay fever are readily absorbed from the conjunctiva.

Nasal Cavity.

Compared with the anterior nares, the posterior are poor in micro-organisms. This is no doubt due to the filtering of the air by the hairs, the tortuosity of the channel causing dust and bacteria to strike the walls where they are held by a moist surface, and the action of the ciliated epithelium in carrying them imbedded in mucus, again to-

ward the anterior nares. Nevertheless, the nasal mucous membrane is a common infection atrium for streptococci, staphylococci, diphtheria and influenza bacilli, the diplococcus of epidemic meningitis, and, probably, for other infectious agents.

Mouth. At least thirty species of micro-organisms flourish in the oral cavity, some of them being pathogenic: staphylococci, streptococci, pneumococci, and often diphtheria bacilli. They are constantly removed with the saliva, and through the extensive desquamation of the epidermis occasioned by mastication. Saliva is not germicidal, but inhibits the growth and weakens the virulence of some bacteria. The fetid breath and the sordidity observed in fevers where the mouth is dry are attributable at least in part to the lack of saliva with its anti-infectious properties. The great rapidity with which wounds of the mouth heal is a potent factor in preventing serious infections.

Lungs. Micro-organisms do not readily reach the ultimate ramifications of the bronchioles. In ordinary respiration the velocity of the inspired air is so reduced as it nears the alveoli that the further movements of the gases is one of gradual diffusion more than of violent admixture. Consequently there are greater opportunities for germs to come in contact with the bronchial walls where they become imbedded in mucus with which they may be expelled by coughing and the action of the ciliated epithelium. Both the alveolar epithelial cells and the leucocytes which enter the air sacs and bronchioles have been shown to take up bacteria. The conditions in the lungs which favor the development of infections, bronchitis, pneu-

monia, influenza, tuberculosis, etc., are by no means clearly understood. Variations in individual resistance, here as in other parts of the body, are certainly of great importance. It is probable that the lung is the infection atrium for a number of our acute infectious diseases. It has been demonstrated that systemic infections, as with anthrax bacilli, may be caused by the inhalation of the germs.

Stomach.

The gastric juice, through the hydrochloric acid it contains, is able to kill anthrax, typhoid, tubercle bacilli, cholera vibrio and other organisms. Clinical and experimental evidence shows that this power is often inadequate, virulent micro-organisms reaching the intestines in spite of it (typhoid, cholera, dysentery, tuberculosis, etc.). It is probable that bacteria in the stomach are often protected against the action of the gastric juice to some extent by being imbedded in solid particles of food. Certain acidophilic germs, as well as yeasts and torulæ, seem to flourish in the gastric secretions; these are largely non-pathogenic, but the regularity with which peritonitis follows perforating wounds of the stomach indicates that it probably always contains pathogenic bacteria, though it may be only their temporary habitat. The gastric juice may render some bacteria harmless by digesting their toxins; one gram of the gastric juice of a dog will neutralize fifty fatal doses of diphtheria toxin, or 10,000 of tetanus toxin, using the guinea-pig as the test animal. On the other hand, the toxin of the bacillus of botulism (causing a form of meat poisoning) seems to be uninfluenced by the stomach contents, as the development of the intoxication indi-

cates. Vomiting is often a means of ridding the stomach of toxic substances, including bacteria. The stomach itself is exceptionally free from infections.

Intestines. The bile is moderately bactericidal for some germs, but, on the whole, the intestinal secretions have low germicidal powers; this is indicated by the fact that the colon contains many more bacteria than the duodenum. On the other hand, the pancreatic juice destroys some toxins (diphtheria, tetanus) more powerfully even than the gastric juice. This ability of the pancreatic juice to destroy toxic bacterial products may explain the more frequent occurrence of enteritis in the ileum than in the duodenum. The bile also has a neutralizing power for some toxins. Although a number of pathogenic bacteria inhabit the intestinal tract (colon bacillus, streptococci, etc.), they do not often set up inflammatory processes in the adult. The tissues become accustomed to their presence. The pathogenic bacteria which do not normally exist in the intestines are those which, on introduction, are most likely to cause disease (typhoid, cholera, dysentery, etc.). The intestinal tract of the infant, on the other hand, is frequently attacked by some micro-organisms (streptococcus, colon bacillus, bacillus pyocyaneus), which in the same locality in the adult appear harmless. The fact that many individuals are not stricken in an epidemic, in which all are equally exposed to infection, points to the probability that pathogenic organisms (typhoid, cholera and dysentery) often traverse the intestinal canal without inducing disease. Naturally, microbes are eliminated in enormous quantities in the feces, and in

inflammatory states this elimination is increased by diarrhea. It is also not to be forgotten that the intestinal tract is, to a considerable extent, a lymphoid organ, and that consequently in the presence of infection enormous quantities of phagocytes can quickly be called into action.

The protective properties of the genito-urinary surfaces are not different in principle from those already mentioned (vaginal acidity, urinary irrigation).

B. THE PROTECTIVE NATURE OF INFLAMMATION.

Nature of Inflammation.

Although there are many chemical and physical agents which may cause inflammation, we are interested here only in those of an infectious nature.

Inflammation may be considered as a reactive condition on the part of the tissues, which develops in response to the action of some injurious agent. The process may be beneficial in some instances, while in others it may be pernicious from the beginning to the end. The thickening of the endothelium of the cerebral vessels as one sees it in syphilis is a progressive, reactive change which in no sense can be of benefit to the individual, and which can have no conceivable function in overcoming the syphilitic infection. Likewise, the new formed connective tissue seen in alcoholic cirrhosis of the liver is of no benefit to the hepatic tissue, though it may serve in some degree to protect the liver cells from the alcohol which continues to be ingested. In an ulcer of the cornea the presence of serum and of leucocytes, as well as the proliferation of connective tissue, may be the *sine qua non* for the healing of the ulcer, yet the resulting scar

may greatly impair the vision. The inflammation in the instances cited is injurious because of the functional importance of the tissues involved. On the other hand, an extensive scar which has formed in tissues of less functional importance, as in the skin and subcutaneous tissue, may be harmless.

It is then to be recognized that there are certain consequences of the inflammatory reaction, the seriousness of which depends on the situation, severity, duration and extent of the process, and that these consequences are independent of any protective function the inflammation may have exercised.

Variations in the Reactions.

The amount and character of the reaction are subject to many variations, depending on a number of conditions:

1. It varies with the nature of the microbe. Non-pathogenic organisms induce little more inflammation than so many minute, inanimate, non-toxic particles. The tubercle bacillus causes especially the formation of connective tissue, giant cells and the accumulation of lymphoid cells, aside from some retrogressive changes characteristic of the disease. Organisms similar to the streptococcus and pneumococcus lead to the formation of pus and fibrin, to the accumulation of serum and of polymorphonuclear leucocytes more than mononuclears, whereas the proliferation of fixed tissue elements is secondary. The tetanus bacillus alone causes almost no local inflammatory change.

2. The reaction is influenced by the virulence of a particular bacterium. A streptococcus which has lost its virulence is disposed of by the animal

tissues with a minimum tissue reaction, perhaps no more than slight congestion and edema and the wandering in of a few leucocytes; one of higher virulence causes an intense reaction, manifested by congestion, edema, hemorrhages, necrosis and pus formation; then streptococci of such great virulence that they destroy life in the course of a few hours are occasionally encountered in wound infections and in peritonitis, having in the meantime elicited a minimum inflammatory reaction.

3. It has a relation to the resistance or the natural immunity of the individual. Metchnikoff, in particular, has shown that animals of high resistance to a particular microbe destroy the germ quickly by phagocytosis (the ingestion of particles by cells, especially the leucocytes), while in susceptible animals the accumulation and activity of phagocytic leucocytes are deficient.

Leucocytes.

The occurrence of leucocytes in inflammatory conditions is so characteristic that one naturally seeks to associate their presence with some influence which is exerted by the toxic substance or the bacteria which cause the inflammation. It is a long-known fact that some microbes attract one kind of leucocyte, that others attract another kind, and that in still other instances the leucocytes appear to be either uninfluenced or actually are repelled by the infecting agent.

Chemotaxis.

This phenomenon of living cells moving toward or away from certain other cells or substances is termed chemotaxis; the former is positive, the latter negative, chemotaxis. There is a somewhat general law, but one to which exceptions exist, that, regardless of the microbe involved, the more

acute the inflammatory process the more do polymorphonuclear leucocytes accumulate, while in the more chronic infections, with much connective tissue formation, the mononuclear leucocytes predominate. Thus in tuberculosis one finds lymphocytes and plasma cells—mononuclears—predominating greatly over the polymorphonuclears. In the acute purulent infections, on the other hand—streptococcus, staphylococcus, pneumococcus—the latter type of leucocyte predominates, the mononuclears being fewer and remaining at a distance from the center of action. There is reason to believe that the mononuclear leucocytes play an important, though perhaps indirect, rôle in the formation of the connective tissue.

Phagocytosis. The ingestion of particles by living cells, phagocytosis, is a property which many cells possess. Although micro-organisms and inanimate particles are sometimes found in epithelial cells, certain of the mesoblastic cells have this function pre-eminently: Polymorphonuclear leucocytes, large mononuclear leucocytes (lymphocytes), ameboid connective tissue and endothelial cells. Of these the polymorphonuclear leucocytes, the microphages of Metchnikoff, have the greatest phagocytic power; the others, the macrophages, are more exceptionally phagocytic. Now, the mere ingestion of the bacteria by such cells would not be of necessity injurious to the microbes; indeed, opponents of Metchnikoff's phagocytic theory of immunity hold that phagocytosis by wandering cells may be, and often is, pernicious, in that the cells may return to the circulation and spread the infection to other parts. But when we learn that after ingesting the bacteria the phago-

cytes are often able to kill and digest them, it is realized that the process may be a genuine protective factor. This being true, the importance of positive chemotaxis in recovery from an infection becomes manifest. It is also represented that phagocytic cells have the power of excreting their germicidal substances into the plasma and serum and lending to the latter a bactericidal power. Furthermore, it is held that they may absorb liquid poisons, bacterial toxins, and in some manner destroy their toxicity. As shown later, these are essential points in the phagocytic theory of immunity.

Influence of Plasma and Serum.

Serum, even when entirely free of leucocytes, has bactericidal powers; it need not be discussed at present whether this power exists primarily in the serum or is one conferred on it by the leucocytes. In view of its presence, however, it is evident that the serous exudate which is usually present in inflammations, especially the acute, may be of influence in combating the infection. Serum often contains natural antitoxins, and, in addition, it may be of value in lessening the toxicity of poisons by diluting them and aiding in their elimination.

Fibrin.

The abundant deposit of fibrin seen in some inflammations is of mechanical value by hemming in the infection and in offering a barrier to the rapid diffusion of toxins. We are all familiar with the part played by fibrinous and fibrous adhesions in preventing a localized peritonitis from becoming generalized. In prolonged inflammations fibrin furnishes a ground substance into which new connective tissue and vessels grow (organization).

Inflammatory Connective Tissue.

The new formed connective tissue seen in many inflammations, especially the chronic, as in tuberculosis and actinomycosis, offers an important barrier to the extension of an infection. Perhaps no better example of this could be cited than the dense tissue which forms around a tuberculous sinus or abscess.

To sum up, the inflammatory reaction antagonizes infections, 1, mechanically, through the formation of new connective tissue around the focus, and dense accumulations of leucocytes and fibrin; 2, through the bactericidal and antitoxic actions of the lymph and serum; 3, through the phagocytic action of ameboid cells.

The value of hot applications in local inflammations, in that they increase congestion, which hastens the exudation of plasma and leucocytes and the proliferation of cells, finds a logical explanation in view of the facts mentioned. Also increase in local temperature probably favors chemical actions. The special features of the phagocytic theory of immunity are considered in a later chapter. For many details in regard to inflammation, the reader is referred to the classic article of Adami on this subject in the first volume of Allbutt's System of Medicine.

C. THE ANTIBACTERIAL AND THE ANTITOXIC NATURE OF NATURAL IMMUNITY.

In a previous page it has been stated that natural immunity may be either antibacterial or antitoxic. We have seen that the protection afforded by the body surfaces may be effective against both microbes and their toxins, and that local inflammatory processes, although most certainly antago-

nizing the bacteria, may at the same time have some antitoxic value.

Natural Antibacterial Immunity.

The term natural immunity, however, as indicated in the first chapter, has a peculiar application to the natural resistance of some species or races of animals to infections to which other species or races are susceptible; and to an unusual individual resistance often seen in members of a given race or species. This condition depends on properties residing in the tissues or fluids of the body, and consequently is independent of any protection which the body surfaces afford. Its presence is demonstrated in the most striking manner by the experimental method, when microbes or toxins are injected directly into the tissues or circulation. At the same time every-day observation provides many examples.

In determining the antibacterial nature of immunity in a given case there are two conditions which must be proved: 1, that the body cells or fluids are able to destroy the microbe, and that this power is sufficiently strong to make it reasonable that the immunity depends on it; 2, that the immunity does not depend on non-susceptibility to a possible toxin of the microbe, nor on a naturally existing antitoxin.

To prove the first condition, three procedures may be resorted to: First, the microbes are injected into the subcutaneous tissue, the peritoneal cavity or the blood vessels. If the animal does not become ill after a dose which, in proportion to weight, is pathogenic for some other test animal, an immunity is indicated. At a proper interval all the tissues and fluids are examined to determine the fate of the microbes. This may be done

by staining the fluids and cells for bacteria and examining with the microscope, or by inoculating culture tubes with the fluids, the growth or non-growth of colonies determining whether or not the microbes have disappeared. Examinations of this nature often disclose the fact that many of the bacteria have been phagocytized by the leucocytes, while others have apparently succumbed to the germicidal action of serum or plasma. It is often desirable to determine the extent to which microbes are eliminated through the excretions (urine or feces); this is best done by the culture method, but is a difficult technical problem.

Second, the animal's serum or plasma may be mixed with a suspension of the microbes in a number of test tubes, using varying amounts of serum with constant amounts of the bacteria in the different tubes, and at a subsequent period, from three to twenty-four hours later, cultures are made from these mixtures to determine the bactericidal power of the serum. The numbers of colonies which appear in these cultures, minus the number which appear when serum is not added, is an index of the bactericidal power of the serum. If this power is found to be high, it is, in the present state of our knowledge, considered as presumptive evidence that the natural immunity of the animal depends on it. It is, nevertheless, a fact that the antibacterial immunity of an animal does not always go hand in hand with the bactericidal power of its serum. A well-known illustration of this is the following: Both the dog and the rat have a rather high degree of immunity against infections with the anthrax bacillus; yet it has been found that the serum of the dog has

almost no bactericidal effect on this microbe, while that of the rat has a very strong effect. At the same time we should remember that the bactericidal power of the serum does not necessarily represent the entire antibacterial function of the body. In the serum we have none of the body cells, and especially none of the phagocytes, the destructive action of which on some bacteria is well known.

Third, it is now possible to perform test-tube experiments with the leucocytes of an animal, whereby the phagocytic power of these cells for a given microbe may be determined. This may be done by counting in stained specimens the number of bacteria which are englobed; or the bactericidal power of the leucocytes may be determined approximately by performing the culture experiments described in the preceding paragraph, in this instance, however, substituting fresh defibrinated blood for the serum. If the bactericidal power of the defibrinated blood (containing leucocytes) is greater than that of the serum alone, the effect of the leucocytes becomes apparent. This receives further consideration in the chapter on phagocytosis.

Alexins.

At a time when the antitoxic action of serums was not appreciated, Buchner gave the name of alexins (from the Greek, ἀλέξειν, to ward off) to the protective substances of the serum, i. e., to the bactericidal substances, making the observation that they were very labile substances, losing their power spontaneously in a few days when exposed to the air and light, or when they were heated to 55 C. for thirty minutes.

Natural Antitoxic Immunity.

In determining the presence or absence of antitoxic immunity, the toxin of the microbe, of

course, must first be in hand. The methods of obtaining toxins will be referred to later. If the animal resists a dose of toxin which, in proportion to weight, produces disease in some other susceptible animal, the tissues or fluids of the first animal may contain antitoxin. It will be indicated later how this result may be obtained even without the presence of antitoxin, the immunity being due to some other obscure cause. If the resistance is referable to the presence of antitoxin, the latter may be detected in the following manner: The animal is bled, its serum collected from the clot, then mixtures of the serum and of the toxin are injected into animals of known susceptibility for the toxin. If the test animal is in this way protected from an otherwise fatal dose of the toxin, it is evidence that the serum contains an antitoxic substance.

Following this method of experimentation, if antibacterial properties are found to the exclusion of antitoxic, the immunity is considered to be antibacterial; and with the converse result it is antitoxic. It is, of course, conceivable that in a given case it might be both antitoxic and antibacterial. In dealing with diseases of which the specific microbe is known and cultivated, the existence of antibacterial or of antitoxic substances can usually be found by the methods described. If the etiology is unknown, as in scarlet fever, measles, syphilis, etc., that is, if the virus and its toxin can not be obtained in quantities, the nature of the resistance is not at present open to determination.

Examples are known in which, in spite of rather high resistance on the part of the animal, its serum contains neither strong antitoxic nor

bactericidal properties. This relationship exists between a number of animals and such bacteria as the pneumococcus, staphylococcus and streptococcus. It is possible that the phagocytes are important factors in immunity to these infections.

It is seldom that natural resistance is absolute. Pasteur found that the great immunity of the chicken for anthrax could be overcome by immersing the animal in cold water, the reduction in body temperature supposedly decreasing the resistance. It was stated in a previous chapter that physical exhaustion, hunger and exposure to cold may also reduce natural resistance. Pestilence and famine often go hand in hand.

Relative Immunity.

Similarly, antitoxic immunity usually is relative. The chicken, which withstands a large quantity of tetanus toxin when injected into the skin, muscles or circulation, succumbs when the toxin is injected directly into the nervous tissue. As an illustration of natural immunity to toxins, the following table serves a good purpose. The horse is the most susceptible animal for tetanus toxin. If the minimum fatal amount for one gram of horse weight is taken as a unit, this scale of resistance for some other animals is obtained (Knorr):

For 1 gram of guinea-pig weight	2 units are fatal
For 1 gram of goat weight	4 units are fatal
For 1 gram of mouse weight	13 units are fatal
For 1 gram of rabbit weight	2,000 units are fatal
For 1 gram of chicken weight	200,000 units are fatal

Non-susceptibility.

In view of the high immunity of the chicken against tetanus, one may be led to suppose that its serum would contain a large amount of antitoxin, yet experiments show that it possesses practically no tetanus antitoxin. This fact suggests that there is a distinct type of natural immunity

which, it is thought, may be independent of both the antibacterial and the antitoxic properties of the body.

Cell Receptors.

It is now thought that the toxic elements of bacteria are chemical substances (very complex, surely) which are able to injure the tissues, i. e., to cause disease, only by entering into chemical union with substances which the cells contain. Such chemical substances of groups pertaining to the cells will be referred to later under the name of cell receptors. Accordingly, if the cells of an animal do not possess groups or receptors which are capable of forming a chemical union with the toxin, the latter would be unable to produce injury, i. e., the animal would be immune even in the absence of all bactericidal or antitoxic properties. This condition, however, is not one which is capable of experimental demonstration, at least at present, but the conditions point irresistibly to its existence in some cases.

We are accordingly led to the conclusion that immunity to toxins is not in all cases antitoxic, in the sense that the serum contains demonstrable antitoxin; and likewise that immunity to bacteria is not in all cases antibacterial, in the sense that the serum contains substances which are able to kill the bacteria in test-tube experiments. Nonsusceptibility and phagocytosis may be of importance in resistance of this type.

Importance of the Tissue Attacked.

There is another factor, however, which may throw light on the type of natural immunity just considered: We know that tetanus toxin causes tetanus through its power of uniting with the nerve cells, and we may consider that tetanus is a very fatal disease, primarily because of the vital

nature of the tissue which it attacks. Now, if the toxin, instead of uniting with the cells of a vital organ, were to combine with cells of less importance to the economy, as, for example, the cells of the subcutaneous tissue, it is probable that we should have no tetanus. In some of the lower animals there is reason to believe that the toxin of tetanus does unite with such tissue (Metchnikoff). Roux and Borrel believe that the greater degree of immunity which the rabbit has over the guinea-pig is due largely to the fact that the rabbit's liver is able to fix a great deal of the toxin. And Metchnikoff has found that the liver of the scorpion, which has an absolute immunity to tetanus, absorbs the toxin and retains it for months.

Summary.

By way of summary, then, we may say that the natural blood immunity and tissue (histogenic, Behring) immunity depend on the following factors: Bactericidal and antitoxic powers of the serum and plasma, the destructive effect of the cells, especially the phagocytes, on both bacteria and toxins; a possible absolute non-susceptibility in some cases (the absolute non-existence of suitable cell receptors); the overwhelming distribution of the suitable receptors for the toxin in organs of less vital necessity for the individual, thus diverting it from more important organs.

In order that a pathogenic organism produce a progressively fatal disease in a susceptible animal, the following obstacles must be surmounted: The strong defenses of the body surfaces must first be overcome; a local inflammatory reaction which may have been excited must first prove itself to be inadequate for the limitation of the infection; there must be an insufficient supply or insufficient

activity of antimicrobic and antitoxic processes in the body fluids and cells.

In view of the wide variations in the nature of different infectious agents, it is possible that the defensive means which would counteract one might be inadequate for another; and inasmuch as animals appear to differ as much in the character of their defensive as microbes do in their offensive powers, there is abundant room for the display of the various phenomena of natural immunity and of natural susceptibility with which we have become familiar.

OTHER PROPERTIES OF NORMAL SERUMS.

Hemolysis. In addition to the bactericidal and antitoxic action of many normal serums, they often possess other characteristics which are of the highest interest in the study of immunity. In earlier days it had been noted that the transfusion of blood from one species to another was often fatal to the injected animal. Later investigations showed that this was due to toxic substances in the transfused blood; substances which, above all, destroyed the red blood cells of the injected animal. This action, in which the hemoglobin is dissolved out of the red blood cells, may be reproduced in test-tube experiments by mixing the blood cells of one animal with the serum of another which is toxic (e. g., rabbit blood + goat serum). This is the phenomenon of hemolysis, and the appearance of such a tube is exactly like that seen when blood is mixed with distilled water or even with tap water; i. e., it is a laking of the blood, it loses its opacity and assumes a beautiful cherry-red color. The serum of practically every species contains a

hemolytic substance (a serum hemolysin) for some kind of erythrocyte.

Cytotoxins.

Some serums also contain toxic agents for other cells; they are generally called serum cytotoxins. The serum of the eel not only contains a strong hemolysin, or hemotoxin, but also a powerful poison for nervous tissue, neurotoxin. Similarly we have normal leucotoxins for leucocytes, nephrotoxin for kidney tissue, etc.

Agglutinins.

Another property of many normal serums is that which causes agglutination or clumping of bacteria, as one sees it in the Gruber-Widal test for typhoid. Even normal human serum may agglutinate the typhoid bacillus, but to a less degree than that of a typhoid patient.

Precipitins.

One serum often causes a precipitate in the serum of another animal, or in a bacterial culture filtrate.

In considering these facts, one becomes conscious of the great complexity of that substance which plays so important a part in immunity and its study—i. e., the blood serum.

CHAPTER VI.

ACQUIRED IMMUNITY.

Immunity which is acquired as the result of infection is said to have been acquired naturally, a very different thing from natural immunity. Immunity which is acquired artificially may be active, as in vaccination; or passive, as when diphtheria antitoxin is injected prophylactically.

Active Immunity.

One who has recovered from scarlet fever, smallpox, plague, typhoid fever, etc., becomes possessed of lasting protection against subsequent attacks. On the other hand, the immunity afforded by an attack of certain other diseases usually is of shorter duration: cholera, diphtheria, pneumonia, etc. So far as known, the acquired protection is very specific in character: e. g., a person who has had measles may still have scarlet fever; or an attack of cholera does not protect against a later attack of typhoid.

In a number of diseases one attack confers no evident protection against a second; gonorrhea, influenza, recurrent fever and malaria. Some diseases may create a predisposition for recurrence: erysipelas, influenza, diphtheria in some instances, although a natural susceptibility of the individual may explain repeated attacks.

A very important factor for progress in artificial immunity was the knowledge that even a light attack of an infection (scarlet fever, cholera, typhoid, smallpox) may be efficient in conferring immunity. Such light attacks are frequently noted sporadically and in epidemics, while occasionally an epidemic is mild in character through-

out. An epidemic of benign smallpox recently prevailed in the middle Western states and the mild character of the plague which was endemic in San Francisco will be remembered. In these instances it seems probable that the mild character of the disease depends on the low virulence of the organism which causes the epidemic; and the condition suggests the possibility of artificial attenuation of virulent micro-organisms for the purpose of inducing at will infections of a benign character.

Vaccination.

It might be possible to so modify the virus that protection could be established without setting in motion the actual disease even in a mild form. An attenuation of this nature had long been practiced with smallpox virus. Before cowpox was resorted to as a source of vaccine, it had been the custom to inoculate the genuine virus of smallpox, for the purpose of producing immunity. Contrary to the natural expectation, this method, instead of reproducing severe smallpox, often caused the modified disease which we call varioloid. This phenomenon may depend on the fact that the virus finds the skin and subcutaneous tissue an unfavorable medium for the development of virulence; a condition which would be equivalent to an attenuation of the microbe. The pathogenicity of the cholera vibrio in animal experiments is affected similarly in subcutaneous injections. It is now generally considered that cowpox is smallpox which has suffered a decrease in virulence because of its passage through the cow. Consequently, when this weakened virus is planted in the skin of man, where it may undergo further attenuation and produce the mildest possible form of modified smallpox, we have an ideal vaccine.

Attenuation.

Passage. In a similar manner the virulence of the anthrax bacillus for sheep may be lessened by passing the organism through the dove. This method of decreasing, or in some cases of increasing, the virulence of a micro-organism is known as passage.

No single method of attenuation is suitable for all organisms. Pasteur found that cultures of the bacillus of chicken-cholera become so weakened when exposed to the action of light and air that they may safely be used as vaccine; also that the anthrax bacillus when grown at 42° C. is attenuated and does not form spores, and consequently becomes a suitable vaccine for sheep and cattle. Of no less interest to us is Pasteur's method of attenuating the virus of hydrophobia by desiccating the spinal cords of infected animals (rabbits); the altered cords are then suitable for the immunization of individuals who have been bitten by a rabid animal.

Work of the past decade has shown that successful vaccination is possible against cholera, typhoid and plague by the inoculation of avirulent cultures, or those which have been killed outright by heat. In so far as we know the immunity which is caused by vaccination or protective inoculation is antibacterial, or, better, antimicrobic. If the cause of the disease is unrecognized, as in smallpox and hydrophobia, there is no means of determining whether it is antibacterial or antitoxic.

Nature of Acquired Immunity. One may ask if acquired immunity to bacteria and to toxins is due to the presence of the antibacterial and antitoxic substances which were mentioned in connection with natural immunity. Although normal serum is strongly bactericidal

for the typhoid bacillus, the serum of one who has recovered from typhoid fever possesses this power to a much greater degree. As this is true in many other bacterial infections, the new resistance is held to depend on the increase of bactericidal substances in the serum. Similarly in acquired immunity to diphtheria and to tetanus, the most conspicuous change is a great increase in the corresponding antitoxins. The result is the same, regardless of whether the immunity be produced by a natural attack of the disease, or by artificial immunization with the specific microbe or toxin. Accordingly it seems probable that acquired immunity in these instances depends on the presence in the serum of an increased amount of properties which, to a certain degree, may be present normally.

The Leucocytes in Active Immunity.

It was stated in the section on natural immunity that the leucocytes, acting as phagocytes and as resorptive cells, seem to be responsible, at least in part, for natural resistance to an infection.

Metchnikoff and his followers have provided us with many observations which are interpreted as showing that the importance of these cells is continued into acquired immunity. These investigators state that in acquired immunity the phagocytes have a much greater capacity for ingesting and killing bacteria and for absorbing and destroying toxins than when the animal is in a state of greater susceptibility. It is also concluded that the serum in active immunity owes its new or more powerful antibacterial, antitoxic and other properties to the leucocytes, which under the influence of the infection have overproduced and excreted these substances into the plasma.

It will appear later that recovery from certain infections (streptococcus, staphylococcus, pneumococcus, etc.) is not characterized by the formation of antibacterial or antitoxic substances. In these instances it seems probable that the temporary immunity, of which recovery is the outward manifestation, is due to destruction of the bacteria by phagocytic cells. This conception seems all the more plausible when we remember the hyperleucocytosis which characterizes these infections.

Passive Immunity.

Inasmuch as it has proved possible by the prolonged immunization of animals with bacteria or toxins to induce a high concentration of antibacterial or antitoxic substances in their serum, it was the natural expectation that if such serums were injected into other animals the latter would thereby be endowed with an increased resistance to the infectious agent against which the serum had special activities (passive immunization). This has been found to be the case with many antibacterial (typhoid, cholera, plague, dysentery, etc.) and some antitoxic serums (diphtheria, tetanus). Unfortunately the protection afforded by the injection of an immune serum is of short duration (from two to several weeks) ; it is as if a foreign substance had been injected, the fate of which is to be eliminated rapidly. This is in contrast to the condition in active immunity in which the protective substances are often formed over a long period by the body cells.

The Leucocytes in Passive Immunity.

The school of Metchnikoff brings the leucocytes into relation with passive as well as active immunity. It is held that the immune serum which is injected is potent, because it stimulates the leucocytes to a greater phagocytic activity in the case

of antibacterial immunity, or to a greater absorption and destruction of toxins in the case of antitoxic immunity.

Opsonins.

Our knowledge of poisons (see Chapter XIV) is as yet so limited that positive statements can not be made as to the part they play in acquired immunity, although it is thought that immunization with some microbes causes an increase in the quantity of opsonins.

Mention may be made here of the well-known but curious phenomenon that resistance may vary with the age of the individual. Typhoid fever attacks the adolescent or middle-aged rather than the very young or very old. Active tuberculosis grows less common in the later decades of life. Then we have what are distinctively the diseases of childhood: after 15 years of age diphtheria, for example, is uncommon. Some of these instances of acquired immunity may be referable to differences in the character of the cell receptors at different ages, while perhaps others are due to a slow immunizing process occasioned by the prolonged presence of non-pathogenic amounts of the proper micro-organisms.

Bacteriolytic Enzymes.

Emmerich and Loew found that many bacteria produce in culture media, as well as in the animal body, substances which apparently act as ferments and which are able to kill not only the bacterium which secretes the ferment, but many others. For example, pyocyanase, the bacteriolytic enzyme of *Bacillus pyocyaneus*, dissolves pyocyaneus, anthrax, diphtheria and typhoid bacilli, the vibrio of cholera, the streptococcus and staphylococcus. These enzymes usually are not toxic, and it is supposed that during the course of an infection they

reach such a concentration in the blood that they destroy the bacteria which produced them, thus bringing about recovery. It is claimed also that they, either during infection or as a result of repeated injection of the ferment, enter into a somewhat permanent combination with the albumin of the body, forming the so-called "immune-proteidin," on which acquired immunity depends.

It is also stated that with "pyocyanase-immune-proteidin" it is possible to so immunize a rabbit that a subsequent (12 days) otherwise fatal dose of the anthrax bacillus is harmless.

Although the effects of these "enzymes" on anthrax and on some other organisms have been confirmed by a number of investigators, their importance in acquired immunity and in the recovery from infections is very doubtful. There is the special objection to this theory that it puts immunity on a non-specific basis; i. e., pyocyanase will protect against anthrax, diphtheria, etc., while, in reality, all our clinical and experimental data point to the high specificity of acquired immunity.

Immune Cytotoxins.

The serum acquires antibodies not only for bacteria and toxins, but also for many other cells and substances which may be used for immunization. There are many immune cytotoxins, such as the serum hemolysins, leucotoxins, neurotoxins, nephrotoxins, etc., which are formed as the result of immunization with the corresponding cells. (See Chapter XIII.)

Immune Agglutinins.

By systematically injecting an animal with a bacterium or with any tissue cell, agglutinating substances (the agglutinins) are formed and may be demonstrated in the serum. Like other anti-

bodies, they are highly specific for the cell used in the immunization. (See Chapters IX and X.)

It has been found that toxins, other than those of bacterial origin, will yield antitoxins by immunization. Such toxins are snake venom, yielding antivenin; ricin, a hemagglutinating toxin from the castor oil bean, yielding antiricin, etc.

Immune Precipitins and The Biologic Test for Species.

Recently what is termed the biologic test for species has assumed prominence. This test may be illustrated: A goat is injected repeatedly with the serum of man. After a number of injections a very minute amount of this goat's serum will cause a precipitate when mixed with human serum, but not when mixed with the serum of any other animal (except, perhaps, that of anthropoid apes). The test is so delicate that when a small amount of old dried human blood is dissolved in salt solution and treated with the goat serum the precipitation will still occur, and in view of this fact, the test has become of medicolegal importance. The wide distribution of this phenomenon among all kinds of animals gives it great biologic significance.

Kraus found that by immunization with certain bacterial filtrates substances are formed in the serum which cause precipitates in the filtrates. It is further interesting that other albumin-containing substances, as egg albumin or milk, will on immunization, yield specific antibodies. The serum of an animal which has been immunized with goat's milk will cause a precipitate in the latter, but not in cow's milk. (See Chapter XI.)

Antiferments.

It has also been possible to obtain specific antibodies for ferments: for the peptonizing ferments

of bacteria, for emulsin, lab, fibrin ferments, etc.

There are, however, very many substances for which serum antibodies can not be obtained; this is true for all substances of known chemical composition, as acids, bases, salts, and for the alkaloids (strychnin, morphin, aconite, etc.)

CHAPTER VII.

TOXINS AND ANTITOXINS.

Ehrlich's Definition of Toxin.

Through Ehrlich the word toxin has come to have a special significance, being applied only to a certain type of toxic substances. Toxins have the following properties (Ehrlich):

1. They are extremely labile substances which occur as secretion products of vegetable or of animal organisms.

2. Their chemical nature is unknown. The impossibility of obtaining them in pure form and their great lability render them insusceptible to ordinary chemical analysis.

3. An analysis of a toxin may be reached at present only through the medium of animal experiments.

4. Immunization with toxins yields antitoxins. It has not been possible to obtain antitoxins for inorganic poisons, glucosids and alkaloids (morphin, strychnin, etc.)

5. In contrast to well-defined chemical poisons, the action of toxins is characterized by a latent or incubation period. That is, following the introduction of a toxin, a certain period of time elapses before toxic symptoms appear, and this period is greater than the time logically required for the absorption of the toxin through the circulation.[1]

The incubation period may be shortened experi-

1. Recent work indicates that the long incubation period of tetanus may depend, at least in part, upon the length of time required for the toxin to reach the ganglion cells through the axis cylinders of the motor nerves.

mentally by the injection of large quantities of toxin, but it can not be eliminated entirely. Snake poison appears to act without incubation period, but it is still to be classed with the toxins, because of its power to cause the formation of an anti-toxin.

6. "The facts make it necessary to assume, as a condition for the poisonous action of toxins, a specific chemical union of the toxin with the protoplasm of the cells in certain organs." . . . "The affinity of other poisons, as the alkaloids, for tissues, depends not on chemical union, but on some such process as solid solution or loose salt formation."

Preparation of Toxins.

The preparation of soluble toxins is relatively simple. It is necessary only to inoculate a suitable fluid culture medium with a culture of the micro-organism, to allow growth to take place for some days at body temperature, then to pass the fluid through a porcelain or some equivalent filter. The soluble toxins usually may be precipitated from the filtrate by some precipitant, as ammonium sulphate, and preserved in a dried state for a long period. Such a precipitate does not represent the toxin in a pure form, but various proteid substances of the culture medium, as well.

The bacilli of diphtheria and tetanus *Bacillus pyocyaneus,* and *Bacillus botulismus,* are the principal micro-organisms which produce soluble toxins.

When the toxins of these organisms are injected into a suitable animal, phenomena similar to those produced by an infection with the organisms themselves are produced. They are in a particular sense specific toxins. Some micro-organisms, however, produce more than one toxin. The teta-

nus bacillus, for example, secretes, in addition to the toxin causing the nervous symptoms of tetanus, another (tetanolysin, or tetanus hemolysin) which has the power to destroy red blood cells. Ehrlich holds that the diphtheria bacillus produces not only the toxin which causes the acute intoxication of diphtheria, but another of long incubation period which may cause paralysis. Cobra poison has at least two toxins, one which attacks the nervous tissues—a neurotoxin—and another which attacks the erythrocytes; the two may be separated by appropriate measures. As previously stated, the serum of the eel has a strong neurotoxin and a hemotoxin.

Secondary Toxins.

Some micro-organisms produce one or more soluble toxic substances, which it is often difficult or impossible to consider as the actual disease-producing elements of these organisms. Concerning a disease which is so well characterized clinically as tetanus, it is not difficult to determine by inoculation experiment whether one has in hand the specific toxin. The proof is naturally much more difficult in relation to streptococci and staphylococci, for example, where the group of symptoms and the pathologic conditions are not entirely unique for the infection. We are by no means certain that the hemolysin or the leucocidin (toxin for leucocytes) of the staphylococcus, or the hemolysin of the streptococcus are the paramount disease-producing toxins of these organisms, although these substances are true toxins.

An important test for the pathogenic significance of a toxin lies in its ability or inability to cause the formation of an antitoxin which is efficient in the treatment of an infection by the

corresponding organism. This is not the case with the toxins just mentioned. However, one should not place too much importance on such a test, for it is quite possible that we are not able on artificial culture media to obtain the toxin in such concentration that the production of an efficient antitoxin is possible.

Intracellular Toxins, or Endotoxins.

There is a large class of organisms the members of which apparently do not produce soluble toxins; such organisms, however, cause highly toxic diseases (e. g., typhoid, cholera, plague). The dead or ground-up bodies of such bacteria are very toxic; also when the germs disintegrate by a process of autolysis or self-digestion the culture medium becomes toxic because of the cell contents which are set free. Such organisms are said to contain intracellular toxins or endotoxins. In infections by them it is supposed that toxic symptoms are produced when a pathogenic amount of the intracellular toxins is liberated by the bacteriolytic action of the body fluids or cells (phagocytes).

Nothing is known of the nature of such toxins. They certainly are very different from the soluble toxins of diphtheria and tetanus, since immunization with them has not as yet resulted in the production of efficient antitoxins. In spite of this fact, however, it is none the less probable that they are the disease-producing constituents of the organisms. Buchner gave the name of "plasmin" to the cell juice which he was able to express from some micro-organisms.

The McFadyen Method.

McFadyen, by grinding large masses of typhoid bacilli which had been rendered brittle by the temperature of liquid air, obtains from this organism a toxic cell juice. The efficiency of the antitoxin

which he is said to obtain by immunization with this material has not been demonstrated practically. It seems improbable that immunization with this "toxin" will yield a serum differing in properties from that obtained by immunization with the living organisms.

Accidental Toxic Substances.

Toxic substances obtained from bacteria by the action of strong chemicals and extracting fluids, may not represent the essential toxic substance of the organism, but perhaps some disintegration product which happens to be toxic.

It is, of course, common knowledge that an antitoxin is the blood serum of an animal, after the latter has been rendered highly immune by repeated injections of the corresponding toxin. The horse is chosen for immunization because of its marked ability to yield antitoxins (diphtheria, tetanus), because of its size, withstanding much loss of blood, and because of the readiness with which it submits to manipulation.

Preparation of Antitoxins.

Manufacturing plants which produce antitoxins and other antiserums on a large scale have splendidly equipped stables, which are kept in the maximum hygienic condition, and from which rats in particular are rigorously excluded.[2] The horses are carefully groomed and fed and given such exercise as will keep them in a healthy condition.

The toxins, in solution, are injected subcutane-

---

2. The importance of this is very great if, for example, horses are receiving injections of some virulent living micro-organism (as the plague bacillus). In this case living micro-organisms reach the general circulation, and a rat having bitten the animal could well contract the plague and be an evident source of danger, not only to other animals, but to the community at large. Even fly-proof stalls are properly instituted in such cases.

ously.[3] Grave and even fatal reactions may follow the first injections, if the toxin has been given in too large doses or in too concentrated solutions. This is especially true when injecting tetanus toxin. It is of great importance first to establish what the Germans call a *"Grundimmunität,"* which means a primary immunity in the animal itself so that the immunization may then be pushed vigorously until the blood contains antitoxin in high concentration. For this purpose it has been found necessary to weaken the first toxins injected. This may be done by heating the toxin solution to 65 or 70 C. for an hour; by adding to it from 0.05 to 0.4 per cent. of the trichlorid of iodin; or by adding a solution of potassium iodid in which iodin has been dissolved (Lugol's solution). High dilutions of the unaltered toxin may also be used. Gradually the virulence and amount of the toxin injected may be increased until finally the full virulent toxin is given in large doses. The increase in dosage must be very gradual. Eventually as much as a liter or more of diphtheria toxin is tolerated.

Attenuation of Toxins.

Following each injection a reaction occurs. With diphtheria the local swelling may be great, and sloughing may occur. Following an injection of tetanus toxin, tetanic symptoms may appear. In either case, there is some loss of weight and often fever, and another injection must not be given until the original weight is regained and the general behavior of the animal indicates that its former healthy condition is re-established.

Several months of such treatment are necessary

3. For the production of antivenin the snake venom is best injected intravenously.

for the production of diphtheria antitoxin in high concentration. At the end of this time blood is drawn from the jugular vein by means of a large trochar to which a rubber tube is attached. The tube leads to a tall glass cylinder holding from one to two liters, and into this the blood is allowed to flow. Six liters may be drawn safely from a horse of average size.[4] The most rigid asepsis is observed in taking the blood. The glass cylinders, appropriately covered to prevent contamination, are then set in a cool, dark place, and after the serum has separated from the clot samples are taken to be tested for their antitoxic value.

Preservatives of Serums.

The serum, in bulk or after being bottled for the trade, is preserved at a low temperature and in the dark, 0.5 per cent. of carbolic acid having been added to insure sterility. The addition of the acid may cause harmless cloudiness in the serum, but does not destroy the antitoxin. Serums may be preserved perfectly in a dried or frozen state.

Many facts of scientific and practical importance have been brought to light through the immunization of animals on a large scale. It has been found, for example, that following each injection of toxin the amount of antitoxin in the blood suffers a reduction, and only equals or rises above the previous amount eight or ten days later. This decrease is explained by assuming that the toxin has, to a certain extent, united chemically with the circulating antitoxin. It indicates also

4. Some horses may be bled as many as forty times without suffering a conspicuous deterioration in health. In time, however, an animal becomes less valuable as an antitoxin producer.

the period at which the horse should be bled in order that the greatest amount of antitoxin may be obtained. It might even be dangerous to draw the blood before this time had elapsed, since some free toxin might still be in the circulation.

It is noteworthy that all horses are not equally good producers of antitoxin. One may yield a serum of three times the value of another, although the two have been treated identically and seem to be equally immune to the toxin.

Another most interesting fact is that, although the blood of an animal may be very rich in antitoxin, he still may have a disproportionate susceptibility to fresh injections of the toxin.

Many of these phenomena have not been explained satisfactorily.

Standardization of Toxins and Antitoxins.

The necessity of standardizing antitoxins so that dosage may be controlled accurately is self-evident. To meet this need the antitoxic unit familiar in practice was devised.

Behring, and also Ehrlich, decided arbitrarily to consider as the antitoxic unit that quantity of a serum which would protect a guinea-pig from 100 fatal doses of the toxin. Ehrlich's original method of testing a serum was to mix different quantities with 10 fatal doses of the toxin and inject each mixture into a guinea-pig of 250 to 300 grams' weight. That quantity of the serum which protected the animal against the ten fatal doses of toxin contained 1/10 of an immunity unit, and from this result the number of units in a cubic centimeter could be calculated. This method involved the use of toxin as the standard by which the value of the antitoxin was measured, and it was found to be unreliable. A toxin degenerates

rather rapidly, retaining at the same time its binding power for the antitoxin; hence two tests made with the same serum two months apart might indicate different antitoxic values for the serum. Also 10 fatal doses of one toxin often required more antitoxin for neutralization than the same quantity of a second toxin. These phenomena are due to the formation of toxids. (See next chapter.)

On account of these sources of error, Ehrlich devised a new method in which a standard antitoxin or test-serum is used as the starting point for the valuation of a new serum. The test-serum used at the Royal Prussian Institute for Experimental Therapy at Frankfurt, of which Ehrlich is the chief, is a dried and powdered serum of such strength that 1 gram contains 1,700 immunity units; i. e., 1/1700 of a c.c. would protect a guinea-pig against 100 fatal doses of a diphtheria toxin.[5] Any other high-grade serum would have answered equally well.

Standard Antitoxins.

The institute keeps in stock a large number of

5. In Germany the various serums are prepared by private individuals or corporations and manufacturers are required to send a sample of every lot of serum intended for the trade to the Frankfurt Institute that its exact value may be determined. Each bottle eventually receives a stamp signifying the value in antitoxin units of the contained serum. Moreover, samples of every lot of serum are retained in the institute, and from time to time these are tested; and when it is found that the samples have degenerated beyond a certain value the order is sent out to call in all serum belonging to the degenerated lot. When a manufacturer thinks the serum of one of his horses has a high value he may draw a small amount of blood from the animal and send the serum to Frankfurt for a preliminary test. If the serum is sufficiently strong he may then bleed the horse freely; if it is weak he will be advised to continue the immunization for a time.

vials, each containing two grams of this dried serum. The air and moisture are exhausted from each vial and the latter is then sealed in the flame. Once in three months one of these vials is broken open carefully and the serum dissolved in 200 c.c. of a solution made up of equal parts of glycerin and 10 per cent. salt solution; hence each cubic centimeter of the solution contains 17 units. During the succeeding three months this antitoxic solution is used in the comparative valuation of new antitoxins; the solution retains its strength unaltered for this period. For individual tests the serum-solution just described is again diluted seventeenfold, so that each cubic centimeter contains one unit. This adds to convenience and accuracy.

The first step in the process is to standardize some diphtheria toxin in which the degenerative changes (toxoid formation) have come to a standstill. This is done by adding so much of the toxin to 1 unit (1 c.c.) of the test serum that an excess of one fatal dose of the toxin remains unbound by the antitoxin.

The quantity of the toxin which gives this result is called the L+dose.[6] The LO dose of the toxin also is determined, this being the amount which is exactly neutralized by the unit of antitoxin. The use of the two doses serves to eliminate subjective errors on the part of the observer. The L+ and LO doses of toxin are then used to determine the value of new antitoxins. That quantity of the new serum which, when mixed with the L+ dose of toxin, causes the animal to die in

6. L=Limes (Limit); + is commonly used to indicate a fatal result.

four to six days, contains 1 unit of antitoxin. If, for example, 1/100 c.c. accomplishes this result, the serum is of one hundredfold strength i. e., 1 c.c. would contain 100 antitoxic units.

For therapeutic purposes, it is desirable to have a serum of high value in order to avoid giving too large quantities. Several diphtheria serums are on the market which have a value of 500 units to the cubic centimeter. It is difficult to immunize above this point.

Purity of Serum.

In addition to the need of knowing the exact antitoxic value of a serum, it should be determined positively that there is no contamination of the serum, and especially that no adventitious toxin (tetanus) is present.

The sterility of the serum is determined by both aërobic and anaërobic cultures, and its freedom from toxins by injecting considerable quantities into animals. The serum should not have more than 0.5 per cent. of carbolic acid as a preservative. A convenient method of determining this point is the injection of 0.5 c.c. of the serum into a white mouse. If more than this quantity of the acid is present the mouse dies.

Similar principles prevail in the standardization of tetanus antitoxin, the mouse being used as the test animal. Unfortunately tetanus antitoxins practically go without standardization in this country. This would seem to be for commercial reasons only, for they may be standardized with a low percentage of error.

That the United States government is attempting to guard the quality of diphtheria antitoxins

on sale in our markets is apparent from the following statement:[7]

"EXAMINATION OF SERUMS MADE BY LICENSED MANUFACTURERS.

"The act of Congress, approved July 1, 1902, entitled 'An act to regulate the sale of viruses, serums, toxins and analogous products in the District of Columbia, to regulate interstate commerce in said articles, and for other purposes,' and the regulations framed thereunder, approved Feb. 21, 1903, imposed upon the director of the Hygienic Laboratory the duty of examining vaccines and antitoxins for purity and potency.

"Accordingly purchases are made for the Hygienic Laboratory from time to time on the open market by officers of the Public Health and Marine-Hospital Service stationed in various parts of the country. The antitoxin is always bought from reliable druggists, who keep the product under proper conditions of light, temperature, etc. Several grades of diphtheria antitoxin made by each licensed manufacturer are bought and sent to the Hygienic Laboratory by mail for the purposes of these tests.

"The serums are tested not only for potency, but also to determine their freedom from contamination by foreign bacteria, and finally to insure the absence of chemical poisons, especially tetanus toxin. Note is made of the physical appearance of the serum, and tests are made to determine whether an excessive amount of preservative has been added.

"A careful memorandum is made of the facts given by the manufacturer, as stated on the label, as to the number of units contained in the package, and the date beyond which the contents can not be expected beyond a reasonable doubt to yield a specific result. Note is also made of the manufacturer's compliance with the law requiring that the product be plainly marked with the name of the article, and the name, address and license number of the manufacturer.

"Delinquencies that occasionally come to light in these examinations are at once reported to the Surgeon General, U. S. Public Health and Marine-Hospital Service, who

---

7. Taken verbatim from Rosenau, "The Immunity Unit for Standardizing Diphtheria Antitoxin," Bulletin No. 21 of the Hygienic Laboratory of the Public Health and Marine Hospital Service of the United States. M. J. Rosenau is Director of the Hygienic Laboratory.

takes the necessary steps requiring the immediate withdrawal of the particular lot of serum from the market and institutes measures to prevent a repetition of similar errors."

"SERUM ANTIDIPHTHERICUM IN THE PHARMACOPEIA.

"The next edition of the United States Pharmacopeia, being the eighth decennial revision, 1900, which is to appear shortly, will contain an antitoxic serum for the first time. The serum will be known officially as antidiphtheric serum or *Serum antidiphthericum,* and the unit will be recognized as that approved or established by the United States Public Health and Marine-Hospital Service.

"The official text, which has been kindly furnished by Professor Remington in advance, will be as follows:

"SERUM ANTIDIPHTHERICUM.

ANTIDIPHTHERIC SERUM. DIPHTHERIA ANTITOXIN.

"A fluid separated from the coagulated blood of a horse *Equus caballus,* Linné, immunized through the inoculation of a diphtheric toxin. It should be kept in sealed glass containers, in a dark place, at temperatures between 4.5° and 15° C. (40° and 59° F.).

"A yellowish or yellowish-brown, transparent or slightly turbid liquid, odorless or having a slight odor, due to the presence of the antiseptic used as a preservative.

"Specific gravity: 1.025 to 1.040 at 25° C. (77° F.).

"Antidiphtheric serum gradually loses its power, the loss in one year varying between 10 per cent. and 30 per cent. Each container should be furnished with a label or statement, giving the strength of the antidiphtheritic serum, expressed in antitoxic units, the name and percentage by volume of the antiseptic used for the preservation of the liquid (if such be used), the date when the antidiphtheric serum was last tested, and the date beyond which it will not have the strength indicated on the label or statement.

"The standard of strength, expressed in units of antitoxic power, should be that approved or established by the United States Public Health and Marine-Hospital Service.

"Average dose: 3,000 units.

"Immunizing dose for well persons: 500 units."

CHAPTER VIII.

THE "STRUCTURE" OF TOXINS AND ANTITOXINS AND THE NATURE OF THE TOXIN-ANTITOXIN REACTION.

Biologic Analysis

Because of the impossibility of obtaining bacterial toxins in pure form, no conception can be gained of their composition in terms of atoms or molecules, although it is convenient to assume that they have some unknown molecular structure. Inferences as to their nature and structure can be gained only by means of the biologic experiment, i. e., their effects on animals and animal cells under arbitrary conditions.

Neutralization of Toxin by Antitoxin.

When a toxin and its antitoxin are mixed in suitable proportions, the mixture becomes nontoxic as the result of chemical union of the two substances; each molecule of toxin has combined with a molecule of antitoxin to form a new nontoxic molecule which may be spoken of as the toxin-antitoxin molecule. It was at one time supposed that antitoxin had the power of destroying the toxin, perhaps by a ferment-like action. In two instances it has been possible to show that this is not the case. Ordinarily toxins are more susceptible to heat than antitoxins, but in the case of pyocyaneus toxin and snake venom the antitoxins are the more susceptible. Wasserman found that when a neutral mixture of pyocyaneus toxin and its antitoxin was heated to a certain temperature the mixture again became toxic, and Calmette made a similar observation concerning venom and antivenin. If the toxin had been de-

stroyed by the antitoxin the solution certainly would not have regained its original toxicity on the application of heat.

Chemical Nature of the Reaction.

The following facts add support to the view that neutralization consists of chemical union between the two substances:

First, neutralization takes place according to the law of multiple proportions, i. e., ten times a given amount of antitoxin will neutralize a proportionate amount of toxin; second, neutralization is more rapid at warm than at cold temperatures; and, third, more rapid in concentrated than in dilute solutions. These are some well-known laws of chemical reactions.

Ferments.

"Emil Fischer has shown that in the ferments, definite atom-groups of special configuration are present which above all else are requisite for the whole phenomenon (of fermentation). Only such substances as possess a group to which the ferment group corresponds, as lock to key, are subject to the action of a particular ferment." This applies to the action of a particular ferment on only one kind of substance.

Haptophores

Having this conception in mind, Ehrlich assumes that union occurs between toxin and antitoxin through a special group of atoms which the toxin molecule possesses, and which fits into, or corresponds specifically to, another group of atoms in the antitoxin molecule. These are spoken of as the binding or haptophorous groups (haptophores) of the molecules. The haptophorous group of the toxin molecule is highly specific since a toxin can be neutralized only by its own antitoxin, and naturally the haptophorous group of the antitoxin molecule must be equally specific.

Toxophore. The toxin molecule contains not only a haptophorus group, through which it unites with antitoxin in one instance or with tissue cells in the production of disease, but also certain constituents in which the specific activity of the substance resides. Toxin is able to work a change in tissue cells after it has combined with them. The toxic property is said to reside in a toxophorous group. The toxophorous and haptophorous groups are parts of the toxin molecule.

Toxoids. It is a peculiarity of toxins that they lose a certain amount of their toxicity in the course of time, although their binding power for antitoxin remains practically unchanged. In the language of the terms which were used above, the toxophorous groups may degenerate or disappear and leave the haptophorous groups intact. Toxins which have undergone this change are-called toxoids.

Further evidence of the existence of toxoids lies in the fact that when used for immunization they cause the formation of antitoxins. This is possible only when the substance is able to unite with the tissue cells; therefore, the non-toxic toxin or toxoid has retained its haptophorous groups.

A toxin entirely free from toxoids has never been observed, since even during the few days required for its preparation a certain amount of degeneration occurs.

Partial Saturation Method of Study. Additional information concerning the nature of toxin has been gained by experimenting with mixtures of toxin and antitoxin, in which the two are present in varying proportions. This is the "partial saturation" method of Ehrlich. Through a vast number of experiments Ehrlich obtained in-

formation which permitted him to estimate that 200 "binding units" are represented in that amount of diphtheria toxin (hypothetically pure) which is exactly neutralized by one antitoxin unit. If the entire amount of antitoxin, i. e., 200/200, is added to the quantity of toxin in question, complete neutralization of the latter, of course, occurs. In case the toxin is entirely pure, 199/200 of the antitoxin unit would destroy all but 1/200 of the initial toxicity; and 150/200, or 100/200, or 75/200, etc., of the antitoxin when added would permit corresponding degrees of toxicity to be demonstrated through animal inoculations. It was found, however, that neutralization did not take place according to this simple scale. The results were complicated, and Ehrlich has found it convenient to express them graphically in the form of a "toxin spectrum" (Figs. 1, 2, 3 and 4). For example, let 199/200 of the antitoxin unit be added to the proper amount of the toxin, 198/200 to another similar amount, 197/200 to another, etc., down to 150/200. In the last mixture, 50 out of the 200 binding units which the toxin possesses are free, and these 50, rather than some other 50, are free because they have less affinity for the antitoxin than the 150 units which were bound. It has been found that those units which first become free have a low degree of toxicity. It was thought that they might have lost their toxophorous groups, i. e., that they were toxoids; and because of their weak affinity for antitoxin they were called epitoxoids. It was found, however, that they possessed a rather constant though low degree of toxicity and that the toxic action was characteris-

The Toxin Spectrum.

Epitoxoids.

Toxon.

tic. Injection was followed by some local edema, then by a long incubation period, and finally by cachexia and paralysis. On account of this characteristic toxic action and the long incubation period, Ehrlich has concluded that the so-called epitoxoid is in reality a second toxin which is secreted by the diphtheria bacillus. This he now designates as toxon[1] in order to distinguish it from that other constituent of diphtheria bouillon, the toxin, which causes the acute phenomena of diphtheria.

Protoxoids.

Let one now add still smaller amounts of the antitoxin unit to the 200 binding units of the toxin. When 149/200 are added it is found that a certain amount of true toxin remains free, the quantity which is unbound being in direct proportion to the amount of antitoxin withheld. Consequently when but 50/200 antitoxin unit is added the amount of free toxin corresponds to 100 bind-

1. The existence or non-existence of toxons has created a great deal of discussion among investigators. The Swedish chemist, Arrhenius, has recently attempted to apply certain principles of physical chemistry to the study of toxins and antitoxins. It is a well-known fact that some chemical substances, when in solution, have the power of breaking up into their constituent parts; thus sodium chlorid breaks up in part into sodium and chlorin, as sodium or chlorin ions or electrolytes. The dissociated sodium or chlorin may then enter into combination with any other suitable substances which may be present. Arrhenius holds that this is the case with the toxin-antitoxin molecule, that it may to a certain extent again break up into separate toxin and antitoxin. He believes that this dissociated toxin is the substance which Ehrlich has been calling toxon. Madsen, who formerly had done much work with toxons, has now joined with Arrhenius in support of the dissociation theory. In spite of the reasonableness of this theory, Ehrlich and his followers continue to uphold the toxon as an independent toxic substance, and have published additional experiments to support their position.

ing units. If true toxin only remained it could then be said that the constitution of this toxin is: toxin 150 and toxon 50. However, it may be found that as 49/200, 48/200, etc., to 0/200 antitoxin unit are added, no increase of free toxin is found, although the antitoxin added has been bound. In this case, the 50 binding units of toxin which have the greatest affinity for antitoxin are non-toxic; i. e., they are toxoids, and since they have the maximum affinity for antitoxin they are called protoxoids.

Syntoxoids.

It has been assumed also that a toxoid may exist which has an affinity for antitoxin exactly equaling that which toxin possesses; this, as yet purely hypothetical constituent, bears the name of syntoxoid.

Figure 1 is a graphic representation of the toxin just described (Madsen). Probably no two toxins have the same constitution. The toxon zone, for example, could well be much larger in one diphtheria toxin than in another.

Proto-, Deutero- and Tritotoxins.

Refinements in experimentation show that even the true toxin is not uniform in its virulence and its affinity for antitoxin. Accordingly a prototoxin, a deuterotoxin and a tritotoxin may be recognized by this same partial saturation method. (See Fig. 2.) For example, it may be found that when a portion of the antitoxin unit, between the limits of 149/200 and 125/200, is withheld, a toxin is left free which is less virulent than that remaining free between the limits of 124/200 and 100/200; and from this point on the new unbound toxin may be still more virulent. The first would be tritotoxin, the second deuterotoxin and the third prototoxin.

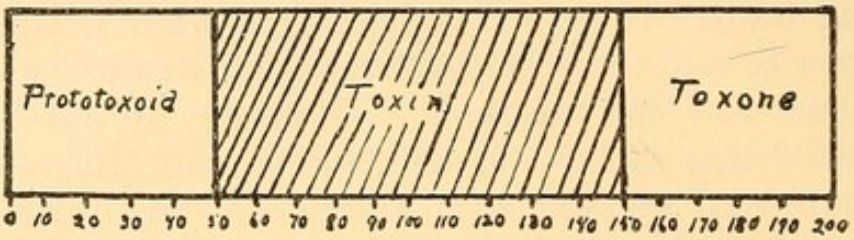


Figure 1.

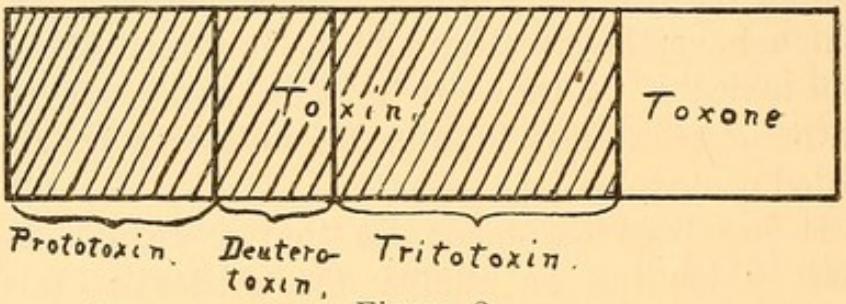


Figure 2.

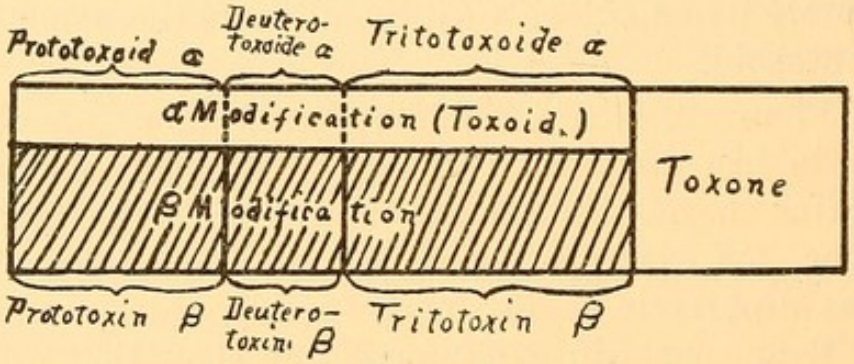


Figure 3.

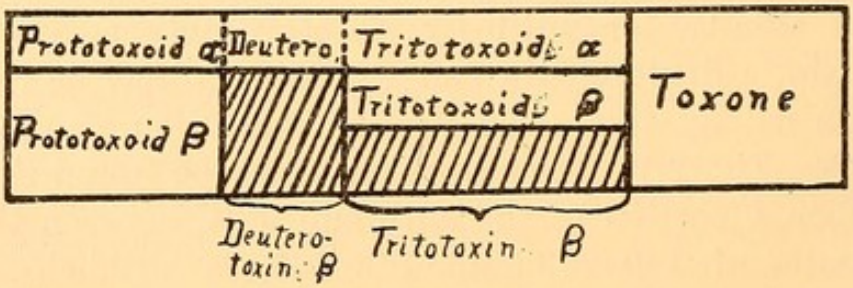


Figure 4.

Figures 1, 2, 3 and 4 are taken from Aschoff's "Ehrlich's Seitenkettentheorie, etc.," Ztschr. f. Allgem. Physiol., vol. i, 1902. Figure 1 is a toxin spectrum worked out by Madsen. Figures 2, 3 and 4 are spectra representing the changes in qualitative and quantitative structure which a toxin may undergo with age, as described in preceding paragraphs.

The "spectrum" of a toxin changes with its age. The prototoxin, and portions of the deutero- or tritotoxin may disappear because of toxoid formation. Such changes have led to the recognition of an alpha and a beta modification of the toxin. The alpha modifications of all three toxins readily become toxoids. Only the beta modification of the deuterotoxin remains constant. The toxon also remains relatively intact (Figs. 2, 3 and 4).

This very complicated method of investigation was also undertaken by Madsen in the study of tetanus toxin, for which a somewhat similar "spectrum" was constructed.

Such spectra have not been worked out in detail for some of the vegetable toxins, as ricin and abrin, but it is known that they form toxoids.

Snake venom differs from the bacterial toxins in structure (See Part II, Chapter III).

The Formation of Antitoxin.

The idea was originally advanced that antitoxin was transformed toxin, a change in the latter having been effected through some action of the tissues. In that case, the amount of antitoxin produced should be roughly equivalent to the amount of toxin injected. This, however, was found not to be the case. A single injection of tetanus toxin may yield 100,000 times the amount of antitoxin necessary to neutralize the toxin injected. An interesting experiment is on record which shows the fallacy of the view just mentioned. An animal, the serum of which was rich in antitoxins, was bled repeatedly until an amount of blood which equaled the total quantity normally present in the animal's body was drawn. Yet the antitoxic power of the new formed blood was practically unchanged.

Metchnikoff, to explain this "overproduction" of antitoxin, has suggested that the toxin molecules may be taken up by phagocytic cells and broken up into an indefinite number of smaller molecules, each of which then is altered in some obscure manner so as to constitute a molecule of antitoxin.

Ehrlich's "Side-Chain" Theory.

The views of Ehrlich have found wide acceptance, and have provided a valuable working hypothesis for many investigations. A consideration of this subject introduces one at once to the well-known side-chain theory of immunity of Ehrlich. It may be considered briefly at this point, in so far as it involves the origin and nature of antitoxin. Ehrlich considers it fundamental, in regard to the metabolic activity of cells, to assume that the cell constituents must enter into chemical combination with food substances in order that the latter may be made available for the use of the cell. It is supposed that cells contain certain atom groups of unknown chemical nature which make possible the binding of food substances. The name of receptor was given to such groups, since substances are received into the cell through them. Inasmuch as the foods and some other substances which penetrate the cells differ in their chemical nature, it is probable that there are various receptors for the various types of substances. The binding, however, is but a preliminary step to profound changes which the substance may next undergo, through the action of other, more vital, cell constituents. That is to say, the receptor is but a link to bring the substance into relationship with the vital activities of the cell, which Ehrlich supposes may reside in a hypothetical *"Leistungskern"* (action center or nucleus).

Receptors.

Multiplicity of Receptors.

Side-Chains.

In view of this conception one readily understands the propriety of considering the receptor as a side-chain of the *"Leistungskern,"* just as the chemist speaks of the various groups which may be at-

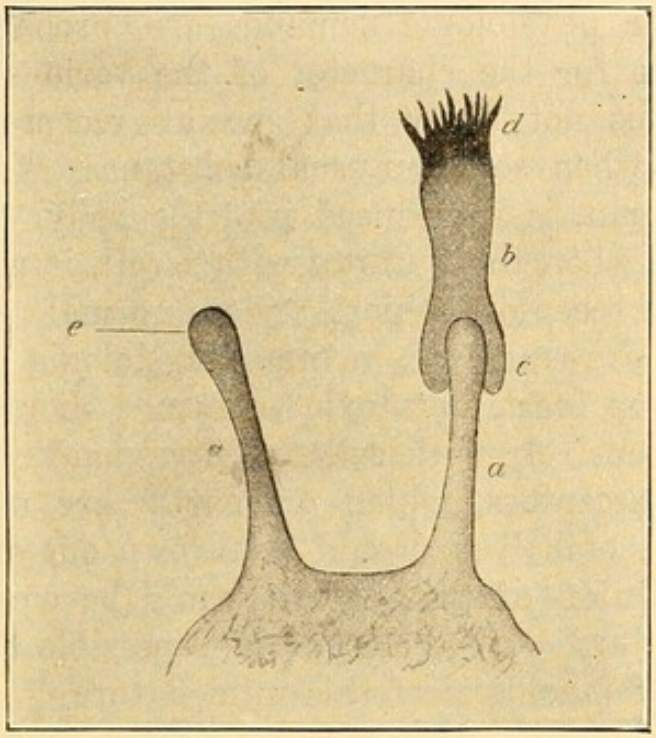


Fig. 5.—Graphic representation of receptors of the first order and of toxin uniting with the cell receptor. *a*, Cell receptor; *b*, toxin molecule; *c*, haptophore of toxin molecule; *d*, toxophore of toxin molecule, *e*, haptophore of the cell receptor. From Ehrlich's "Schlussbetrachtungen," Nothnagel's System of Medicine, vol. viii. This cut is not to be taken as representing the actual morphology of toxins or cell receptors. Nothing is known of their morphology, if, indeed, they have any. The cut is intended merely to represent, in a graphic manner, the supposed chemical structure and mode of action of these substances. This statement applies also to Figures 6 and 7.

tached to the benzol ring, or benzol nucleus, as side-chains (See Chapter XV).

Action of Toxins.

In preceding pages it has been emphasized that a toxin, in order that it may injure a cell, must enter into chemical combination with its constitu-

ents, and it is a fundamental tenet of the Ehrlich theory that this union is one which takes place between the toxin and a cell receptor (side-chain). The cell receptor, then, either is a haptophore or possesses a haptophore as a part of its complex.

As the physiologic demands are probably responsible for the character of the various receptors, it is not likely that special receptors are created when some unusual substance, as a bacterial toxin, is introduced into the body. Consequently, when toxin unites with a cell, it probably occupies receptors which, under normal circumstances, are employed in some physiologic process.

If some inert, non-toxic substance should combine extensively with cells, a corresponding number of receptors, which ordinarily are used for normal metabolism, would be thrown out of function. Union of this nature would be equivalent to an injury of the cell, and it is possible that the action of toxoids is of this mild nature.

When toxin unites with cells there is involved not only the diversion of cell receptors from their customary functions, but in addition the destructive action of the toxin on the vital parts of the cell (perhaps on the *"Leistungskern"*). The more toxin introduced, the greater the number of cell receptors bound, and the greater the injury to the cell.

Hypothesis of Weigert.

In case a non-fatal amount of toxin has been bound, but sufficient to cause some injury, how does the cell respond to the injury? Weigert, a few years ago, gave expression to a hypothesis which is held to have some bearing on this question. In studying regeneration following injury he concluded that tissues have the tendency to

reproduce not only to the extent of making good the injury, but that an excess of new tissue results. The clearest example of this occurrence is that of scar formation, in which a seeming excess of new connective tissue cells is formed, which later disappears in part. Similarly, when a non-fatal amount of toxin unites with the receptors, a cell defect or injury is created. The cell has for practical purposes lost so many receptors. This loss affects the vital activities of the cell, the "*Leistungskern*," and new receptors, identical with those occupied, are reproduced. Following the law stated, they are reproduced in excess of the number injured, and the excess may be so great that the cell may be overfilled with them—so overfilled that many are discharged and reach the general circulation. These cast-off receptors, or side-chains, still retaining their power of uniting with toxin, constitute our antitoxins. As Behring has stated it, the receptor, when attached to the cell, is the agent through which the latter is attacked, but when cast off from the cell becomes its protector (Fig. 5).

Overproduction of Side-chains.

As regards the structure of the antitoxin (cast-off receptor), it is necessary to assume only the presence of the proper haptophorous group. Ehrlich designates all receptors of this simple type as "receptors of the first order." In following sections we will have to do with receptors of the second and third orders.

Receptors of the First Order.

Wassermann gives the following list of antitoxins:

ANTITOXINS FOR BACTERIAL TOXINS.

Diphtheria antitoxin.
Tetanus antitoxin.

Botulism antitoxin.
Pyocyaneus antitoxin.
Symptomatic anthrax antitoxin.
Antileucocidin, an antitoxin for the leucocytic poison of the staphylococcus.
Antitoxins for the blood dissolving toxins of a number of bacteria.

ANTITOXINS FOR ANIMAL TOXINS.

Antivenin for snake poison.
Antitoxin for scorpion poison.
Antitoxin for spider poison.
Antitoxins for certain poisons of fish, eel serum. salamander, turtle, and for wasp poison.

ANTITOXINS FOR PLANT TOXINS.

Antiricin, for a red blood corpuscle poison of the castor oil bean.
Antiabrin, for a similar poison of the jequirity bean.
Antirobin, for robin, a locust tree poison.
Anticrotin, for crotin, a toxin from the bean of *Croton tiglium,* the croton oil bean.
Hay fever antitoxin, for the toxin of pollens which cause hay fever.

ANTIFERMENTS.

Antirennet.
Antipepsin.
Antitrypsin.
Antifibrinferment.
Antiurease, for urease, a urea splitting ferment.
Antilaccase.
Antityrosinase.
Antisteapsin.
Antiferments against the ferments of bacterial cultures.

The above are true antitoxins. There are other substances, however, which occasionally exert an antagonistic action on toxins, although they probably are not true antitoxins. For example, it has been found that cholesterin neutralizes the action of tetanolysin, the hemolytic toxin of the tetanus bacillus.

The discovery of Hektoen that certain salts are able to neutralize the toxic action of some serums, by combining with the so-called complement, may also be mentioned in this connection.

CHAPTER IX.

THE PHENOMENON OF AGGLUTINATION.

Agglutination, in the bacteriologic sense, refers to the clumping and sedimentation of a homogeneous suspension of micro-organisms by the action of a serum.

Specificity. Although a number of investigators had observed the phenomenon of agglutination, Gruber and Durham first saw its significance. They found that the reaction was a specific one, i. e., that the serum which would cause the strongest agglutination of a micro-organism was that of an animal which had been made immune to it by repeated injections.

Widal and Grünbaum. Widal's service consisted in the utilization of the phenomenon as an aid in the diagnosis of typhoid fever. He is the originator of clinical serum diagnosis. It is perhaps largely a matter of accident that we speak of the Widal reaction rather than the Grünbaum reaction. Grünbaum was carrying on the same work at the same time, but Widal preceded him in the publication of his more extensive work.

Normal Agglutinins. In the chapter on natural immunity it was stated that normal serums often are able to agglutinate bacteria. Normal human serum may agglutinate the typhoid, colon, pyocyaneus, and dysentery bacilli, and occasionally the staphylococcus and cholera vibrio; it does not agglutinate the streptococcus and some other organisms. In certain cases it may agglutinate the typhoid bacillus

even when the serum is diluted to one in thirty, a point of practical importance in the clinical use of the test. When a normal serum is found to have a high agglutinating power, a previous infection by the micro-organism is to be thought of. This possibility receives emphasis from the fact that the serum of a new-born child is devoid of many of the agglutinins which are found in later life. Hence, of the so-called normal agglutinins, many, after all, may be acquired properties.

Immune Agglutinins.

The term immune agglutinin is applied to the agglutinating substance in a serum, when the property has developed as a result of infection, or of systematic immunization with the organism. They are formed during infections with the organisms of typhoid, cholera, dysentery, plague, etc.

For the artificial production of agglutinins, the bacteria may be injected into the veins, subcutaneous tissue, or peritoneal cavity; in some cases they may be fed to animals, rubbed into the skin, or sprayed into the lungs. If certain micro-organisms are sealed up in a collodion sac and placed in the abdominal cavity of an animal, an agglutinating serum will be formed; the necessary substances diffuse through the sac and reach those body cells which produce the agglutinin. It is not necessary that living bacteria be injected; in fact, the strongest agglutinin is said to be formed by the injection of bacteria which have been killed by a temperature of 62 C. In certain instances agglutinins are produced by immunization with disintegration products of bacteria or with bacterial extracts.

Agglutinin Producing Organisms.

Nearly all bacteria, even when non-pathogenic, will give rise to agglutinating serums when injected; but not all have the power equally. Nicolle

and Trenell distinguish three groups of bacteria in regard to their agglutinability by the homologous antiserums.[1] The first group includes easily agglutinable organisms, for the most pathogenic: Typhoid, dysentery, cholera, plague, glanders, and the colon, psittacosis, pyocyaneus bacilli, and *B. enteritidis*. They yield agglutinating serums readily either as a result of infection or by immunization. The second group comprises organisms which, during infection or convalescence, do not cause the formation of agglutinins, but may be forced to do so by systematically injecting them into animals. In the third group are included those which, even during prolonged immunization, rarely cause the formation of agglutinating serums: the Friedlander bacillus. These facts may be taken as an index of the diseases in which we may expect to obtain the agglutination reaction by the serum of the patient.

Variations in Agglutinogenic Power of Organisms.

The degree of agglutinating power which may be obtained by immunization varies greatly. Van der Velde speaks of a typhoid serum which in a dilution of one in one million was agglutinating, and Durham had a cholera serum which was effective in a dilution of one in two millions. Such powerful serums are rarely obtained.

Even two different strains of the same organism may differ in their ability to cause the formation of agglutinins. It is generally said that a typhoid strain, which is agglutinated with difficulty, gives rise to a weak agglutinating serum, while an easily

1. The homologous organism for a typhoid serum, for example, is the typhoid bacillus, and vice versa; other organisms, or other serums, are heterologous. These are commonly used terms.

agglutinable strain gives a strong agglutinin. The logic of this will become apparent when we consider the nature of the bacterial substance which causes the body to produce agglutinin.

Variations in Quantity of Agglutinin.

That the agglutinating power of the serum of a typhoid patient varies from day to day is a fact of practical importance. It may be thirty times as strong one day as the next, and may even disappear entirely for a day or two. Hence the importance of making more than one test in a suspicious case, when the first trial has been doubtful or negative. There is no adequate explanation for this great variation. It is said that mixed infections, intestinal hemorrhage, or a sudden pouring out of typhoid bacilli into the circulation may cause a reduction in the agglutinating power. This occurrence has an important bearing on the possibility of using the agglutinating power of the serum as a prognostic sign. Although it has often been noted that in fatal infections agglutinins may be absent from the serum, the variations just mentioned indicate that prognosis could not be based safely on the result of a single agglutination test.

Distribution of Agglutinins in Body.

The agglutinating substance is found in the highest concentration in the blood serum, but it may be demonstrated in the various body fluids and in extracts of the organs; it is said to be particularly rich in the milk. It is present in the serum of an artificially produced blister, and it has been recommended that blistering be resorted to in order to obtain serum for the test. The bile often agglutinates the typhoid bacillus, but the power has no necessary relationship to a pre-existing infection; it is possible that the agglutination in this case is due to obscure chemical causes

rather than to the usual serum agglutinin. The administration of pilocarpin causes a rise in the agglutinating power of the tears, sputum and some other body fluids; the drug increases cell secretions.

Inheritance.

When typhoid fever occurs during pregnancy, agglutinins may appear in the serum of the fetus. On the one hand it has been held that agglutinin passes from the mother to the fetus, or, on the other hand, that the presence of agglutinins arises from infection of the fetus itself.

Although the milk may be very rich in agglutinin, it is doubtful if the serum of a breast-fed child undergoes much increase in its agglutinating power because of the ingestion of the milk. The intestinal juices (trypsin) digest agglutinins.

The origin of agglutinins in the animal body is not known.

Agglutination and Immunity.

One of the most interesting and important phenomena in the study of immunity is the so-called Pfeiffer reaction. An animal which has been rendered immune to cholera by repeated injections of cholera vibrios has the power of digesting or dissolving the latter when they are placed in the fresh serum or in the peritoneal cavity of the immunized animal. Gruber and Durham were studying this phenomenon in the test tube when they first observed the agglutination reaction. It was found that the agglutinating property, as well as the bactericidal power, was the result of immunization. Inasmuch as an increase in the bactericidal power of a serum points to the existence of an acquired immunity, the question naturally arises: Does the associated property of agglutination have a similar significance?

Many observations indicate that the two activities are distinct; that they depend on different substances in the serum. The following are the important points involved:

1. The bactericidal power is destroyed at 56 C., while agglutinins resist a temperature of 62 C.

2. In certain cases it has been possible to cause the bacteria to absorb the agglutinin from the serum, leaving the bactericidal substance intact.

3. A serum may be bactericidal, but not agglutinating.

4. During the course of natural or experimental typhoid fever or cholera the development of the agglutinating and bactericidal powers may not be parallel. In cholera, the agglutinating power may disappear soon, but the bactericidal power remains for a long time.

5. Micro-organisms which have been killed by a bactericidal serum may lose their toxicity; agglutinated bacteria remain virulent.

Besredka found an apparent relationship between agglutination and immunity; if typhoid bacilli were agglutinated before they were injected into the abdomen of a guinea-pig the animal would recover, but if they were not agglutinated death resulted. The explanation offered for this loss of virulence is that the bacilli being agglutinated and immobilized are more readily taken up by the phagocytes; if phagocytosis is inhibited by some means the agglutinated organisms are found to be still virulent.

Koch has attempted to use the agglutination test with the tubercle bacillus as an index of immunity against tuberculosis. This is not accepted as a reliable test for the immunity, but is perhaps

a general index of the ability of the individual to form antibodies for this organism. This method was devised inasmuch as the bactericidal action of a serum on the tubercle bacillus is not readily determined.

Technic of the Agglutination Test.

One may use two methods of determining the agglutination of bacteria: 1. The macroscopic or naked eye observation of the clumping and sedimentation of a homogeneous suspension of the bacteria in test-tubes; 2, the microscopic observation of the clumping of the organisms when the latter are mixed with serum and mounted as a "hanging-drop" preparation.[2]

The Bacterial Suspension.

When the organism to be tested grows rapidly, it is the custom to use a young culture, one which has grown on an agar surface or in bouillon for from eighteen to twenty-four hours. Older cultures of the typhoid bacillus or of the cholera vibrio are agglutinated with more difficulty than a young culture. If an agar culture is used, the bacteria may be washed from the surface by pouring five or ten cubic centimeters of physiologic salt solution into the tube and shaking vigorously; the resulting suspension is then ready for use. For either the macroscopic or microscopic test it is absolutely essential to have a homogeneous suspension of the bacteria, in order to avoid misinterpretations which may be occasioned by the accidental

---

2. For a hanging-drop preparation it is necessary to have a slide with a saucer-shaped depression on one surface. A drop of the solution to be examined is mounted on a cover-glass, and the latter is then mounted, drop side down, over the depression and the edges of the cover-glass sealed with vaselin or paraffin. There is ample room for motile organisms to swim about in such a preparation, and the loss of motility incident to agglutination is readily observed.

or natural clumping of some of the organisms; the tubes should be shaken thoroughly before the emulsions are used. This uniformity of suspension is readily accomplished with such organisms as the typhoid bacillus and cholera vibrio, motile organisms, but when they grow in chains (streptococcus) or in coherent masses (diphtheria and tubercle bacilli) more violent measures must be resorted to. Daily shaking of a liquid culture of the diphtheria or tubercle bacillus is fairly effective, but the medium must be passed through a paper filter before it can be used safely; in this way the larger clumps are removed. Some investigators dry a large quantity of tubercle bacilli, grind them up thoroughly in an agate mortar and suspend the particles in salt solution; the fragmented condition of the organisms does not interfere with their participation in the reaction. One should have a uniform technic in preparing a bacterial emulsion in order to obtain as nearly as possible the same number of bacteria in a given volume of solution, on different occasions. For example, one may uniformly suspend a twenty-four-hour agar culture in ten cubic centimeters of salt solution. A uniform technic makes it possible to observe the quantitative relationship which exists between the mass of bacteria to be agglutinated and the agglutinating power of the serum.

To Obtain Serum.

To obtain serum for the test one may resort to blistering; place a cantharides plaster from one-half to three-fourths of an inch square on the abdominal skin, protect it with a dressing, and in about twelve hours remove the serum with a sterilized hypodermic syringe. Or, one may collect in a small test tube .5 to 1 c.c. of blood from the lobe

of the ear or finger-tip, and draw off the serum after it has separated by clotting. It is the custom in some well-equipped laboratories to fill several U-shaped capillary tubes with blood from the lobe of the ear and to separate the blood from the serum at once by centrifugation. The custom of drying a few drops of blood on a coverglass or on filter paper, and of sending this preparation to a laboratory for the agglutination reaction, has been practiced quite extensively, and is a justifiable procedure when it is not possible to collect the pure serum. It has the disadvantage that the experimenter never knows exactly how much blood has been collected, and consequently can not perform the test with exact dilutions of the serum, the importance of which will be pointed out below. The red corpuscles and débris in such a preparation also interfere with the clearness of the field in microscopic examination, a difficulty which may be partly overcome by filtering the dissolved serum.

Serum Dilutions.

When only a small amount of serum is available, it is necessary to use the microscopic method. Normal human serum, when concentrated, and even when diluted to one in ten or higher, sometimes agglutinates the typhoid bacillus and some other organisms; the same serum, when diluted to one in forty or one in sixty, may not agglutinate. The serum of a typhoid patient, however, or of a typhoid convalescent rarely fails to agglutinate in these higher dilutions. It is generally held that a dilution of one in forty or fifty is sufficiently high to eliminate the possibility of agglutination by a non-typhoid serum, and sufficiently low to render the serums of all, or nearly all, typhoid patients agglutinating. The necessity for dilution of

the serum is emphasized by the additional fact that infections with related organisms, as the colon bacillus, cause a slight increase in the agglutinating power for the typhoid bacillus along with a relatively large increase of colon agglutinins. A test with a low dilution of this colon serum might give a positive reaction with the typhoid bacillus and lead to an incorrect interpretation; but if a dilution of one in forty were used, the non-agglutination of the typhoid bacillus would speak against a typhoid infection. This will be considered under "group agglutination" (Chapter X).

The "Loop" Measurement.

A convenient method of measuring small amounts of culture and serum is by means of a fine platinum wire which is bent at its tip to form an eyelet or "loop."[3] If one places one loop of serum into a small watch glass or hollow-ground slide, and adds nine loops of bouillon or of salt solution, a dilution of one in ten is reached. Five loops of this mixture with five of the diluent gives a dilution of one in twenty. One loop of the second dilution, to which is added one of the culture suspension, gives the desired dilution of one in forty. The last may be mixed directly on the coverglass, and then inverted on a hollow-ground slide. It is readily seen how with even a minute quantity of serum, one may make the test with dilutions of one in ten, one in twenty, one in thirty, one in forty, etc., details which are necessary for a correctly performed test. It is important that in the different dilutions the same amount of bacterial emulsion be used.

---

3. Pfeiffer introduced a conventional "loop" of such dimensions that it holds 2 milligrams of bacterial cells as they are taken from a solid surface, like that of agar.

In the macroscopic test, more serum is necessary, though the quantity need not be large, and the dilutions are made in test tubes of suitable size. One should always deal with definite quantities of the serum dilutions, and should always add the same amount of bacterial emulsion in the various tubes involved in a test.

The Microscopic Reaction.

If agglutination occurs in the microscopic preparation described above, one sees, with the high power, in the course of from fifteen minutes to a half-hour, that two or more micro-organisms which come in contact have a tendency to remain in this position. In the case of a motile organism (typhoid) the movements may be exaggerated for a time. In the course of the next few hours, other cells are added to incipient groups and new groups originate. Motility becomes less and less and eventually ceases, in a characteristic reaction. The maximum change has taken place in from six to eight hours. Not less than four or five cells which are permanently agglutinated are considered indicative of a positive reaction; the test is most decisive when large masses are formed, so large that they are seen readily with a low magnification. A similar preparation to which no serum has been added should always be made, in order to eliminate spontaneous or "auto-agglutination" as a possible source of error.

The Macroscopic Reaction.

In a macroscopic test, the uniform cloudiness of the mixture of serum and bacteria becomes changed by the formation of smaller and larger flakes or clumps of bacteria, which in the course of a few hours sink to the bottom as a white precipitate, leaving a clear overlying fluid. Here also a

control tube, to which no serum has been added, should be preserved for comparison.

The body temperature, which may be obtained in a thermostat, facilitates the reaction.

The Agglutinin Unit.

The value of an agglutinating serum can not be expressed in units with the exactness that is attained in measuring diphtheria antitoxin for the following reasons: 1, The limits of the reaction are not sufficiently definite; 2, a given mass of bacteria has the power of absorbing varying amounts of the agglutinating substance, depending on the concentration of the latter; and 3, it is impossible to obtain standard bacterial emulsions.

One may arbitrarily decide on a unit similar to that of Züpnik, in which a serum which is able to agglutinate a given mass of bacteria in a dilution of one in forty is taken as the standard. If a similar amount of a serum agglutinates in a dilution of 1 in 120 it is said to be of threefold strength.

The value of the agglutination reaction as a clinical diagnostic aid will be considered later in connection with the individual diseases.

Agglutination of Red Blood Corpuscles.

A consideration of agglutination would be incomplete if one did not mention the phenomenon as it occurs with cells other than those of bacteria, in particular the red blood cells. The serums of many animals, as stated in a previous chapter, are toxic for the erythrocytes of some other species. In some instances, the corpuscles lose their hemoglobin under the influence of the serum (hemolysis); in other instances, or even with the same serums, the corpuscles are thrown into clumps and settle to the bottom of the test tube, leaving a clear overlying fluid. The analogy with the bacterial

agglutinins goes still further, in view of the fact that the formation of these "hemagglutinins" may be induced artificially in the body of an animal by the injection of erythrocytes from another species. An animal does not form agglutinins for its own cells (auto-agglutinins), and rarely, if ever, for the cells of another member of the same species (iso-agglutinins). What is said in the next chapter concerning the specificity of the bacterial agglutinins also holds for the hemagglutinins.

Plant Hemagglutinins.

Certain plant toxins, true toxins with haptophorous and toxophorous structures, agglutinate red blood cells: ricin, abrin, crotin, etc. Some of the earliest and most important work which Ehrlich has done in the field of immunity was accomplished with these plant toxins.

CHAPTER X.

THE NATURE OF THE SUBSTANCES CONCERNED IN AGGLUTINATION.

Terms.

Two substances are concerned in agglutination: one, the active or agglutinating substance, exists in the serum, while the other, the substance acted on or the agglutinable substance, is present in the bacteria. The agglutinable substance is generally supposed to be passive in the reaction, while the agglutinating property seems to possess a ferment-like element, which acts on the agglutinable substance. Agglutinin, the term used in the preceding chapter, is now generally applied to the substance in the serum. Recently the bacterial constituent has been called agglutinogen, because of the belief that the agglutinable substance, when introduced into the animal body, stimulates the latter to the formation of agglutinin; hence aggutinogen means, not agglutination-producing, but agglutinin-producing. These shorter terms will be used for the sake of convenience.

Agglutinogen.

The presence of agglutinogen in an organism may be demonstrated in three ways: 1. The mere fact of its agglutinability by a serum is evidence of the presence of an agglutinable substance. 2. If during infection or immunization the serum acquires agglutinating properties, the bacterium possesses an agglutinogenic substance. 3. If a culture is mixed with a serum containing the specific agglutinin, and after a period of contact is removed by centrifugation, the resultant disappear-

ance of agglutinin from the serum, which may be demonstrated, shows that something in the bacteria (agglutinogen) has combined with the agglutinin.

Distribution of Agglutinogen.

The location of agglutinogen in the bacterial cells has received some discussion. There is a tendency to believe that it exists in the cell envelope or perhaps on its surface. It appears to be formed in the cell, and, in some cases, it may be excreted into a surrounding medium; certainly when bacteria die and disintegrate agglutinogen is liberated. The filtrates of certain cultures (entirely free from bacterial cells), when injected into animals, will cause the formation of agglutinins. Also, just as a micro-organism is able to absorb agglutinin from the corresponding antiserum by a process of chemical union, so a filtrate of the type mentioned is able to neutralize the agglutinating power of the serum. In these instances agglutinogen becomes free as a consequence of disintegration of some of the bacterial cells.

The Precipitation Reaction.

The filtrates of certain cultures exhibit another phenomenon when they are mixed with their specific antiserums; this has to do with the bacterial precipitins of Kraus. If, for example, the filtrate of an old typhoid bouillon culture is mixed with antityphoid serum, a distinct precipitate is formed which eventually settles to the bottom of the tube. This is a specific reaction, and does not occur if the filtrate is mixed with some other immune serum. It is thought by some that this so-called precipitable substance in the filtrate is identical with the agglutinable substance (agglutinogen), but this point is still the subject of investigation.

Agglutinogen may be extracted from micro-

organisms by chemical processes. The presence of the substance in the extracts becomes manifest when immunization with them causes the formation of an agglutinating serum. This, again, is the "test of immunization."

Multiplicity of Agglutinogens.

The agglutinogen of one bacterium is not identical with that of any other. If they were identical, immunization with the one would yield an agglutinating serum of equal power for both cells; this, however, is not the result obtained. On the other hand, the agglutinins of two different organisms may coincide to a certain degree, as will be shown under the subject of "group agglutination." Certain experiments go to show that the agglutinogen of even a single micro-organism is not uniform substance. One portion is heat-susceptible, being destroyed at 62 C., while another portion is said to resist a temperature of 165 C. Such technical questions continue to be investigated.

Agglutinogens are said to pass through semipermeable membranes, while agglutinins do not.

Flagellar and Somatic Agglutinogens.

Smith and Reagh distinguish two kinds of agglutinogen in those bacteria which possess flagellæ, one peculiar to the cell body, and the other to the flagellæ.

Properties of Agglutinins.

Agglutinin may be precipitated completely from a serum by the sulphates of magnesium or ammonium, when the salts are used in proper concentrations. Because of their reaction to such precipitating agents, agglutinins are thought to belong to the globulin fraction of serums; whether globulins or not, they are precipitated with them.

Agglutinins resist digestion with pepsin and papayotin, but are destroyed after prolonged exposure to the action of trypsin. An agglutinating

serum which is dried and kept free from moisture and the action of light retains its power unaltered. Similar to agglutinogen, agglutinin is thought not to be a uniform substance, one portion being susceptible to heat, and another portion resistant; these have been called alpha and beta agglutinins.

Structure of Agglutinogen.

It is convenient to speak of the reaction between agglutinin and agglutinogen, and of the process in the body through which agglutinins are formed, in terms of the side-chain theory. Accordingly, if that constituent of micro-organisms which we have termed agglutinogen is the substance which stimulates the tissues to form agglutinin, we must assign to it a haptophorous group through which it may unite with the receptors of the tissue cells. This haptophore comes into play again in the union between agglutinogen and agglutinin, which precedes agglutination. There is no reason for assigning to agglutinogen any other structure than this single haptophore; it is a passive body, similar to antitoxin, and has no other function than that of uniting either with cell or with agglutinin.

Structure of Agglutinin.

Agglutinin also must have a haptophorous or binding group, inasmuch as it enters into combination with agglutinogen. In addition to this binding group, experiments have shown that agglutinin possesses a toxic constituent, which is analogous to the toxophorous group of the toxin molecule. In this case, however, it is called a zymotoxic, zymophorous or agglutinophorous group; supposedly it has a ferment-like activity (Fig. 6). The analogy with toxins goes further, in that the zymotoxic group of agglutinin may degenerate or may be destroyed, leaving the haptophorous group with its binding power for agglutinogen practi-

Zymotoxic Group.

cally unaltered; these are agglutinoids, just as toxins when changed in a similar way are called toxoids. A serum which is rich in agglutinin may be changed into one rich in agglutinoid by exposure to a temperature of from 60 to 75 C., and by the action of acids or alkalies; the change also takes place spontaneously in the course of time, when the agglutinin is in solution. **Agglutinoids.**

Agglutinoids are detected by methods analogous to those used in the recognition of toxoids. If toxoids unite with all the antitoxin in a solution, there naturally remains no antitoxin to unite with true toxin which may be added subsequently. Similarly, if all the agglutinogen in a mass of microorganisms has united with inactive agglutinoid, agglutinin which is added subsequently would have no point of attack and the reaction of agglutination would not occur. So we may say that when bacteria are treated with a serum which has lost its original agglutinating power, and the bacteria are thereby made insusceptible to the action of a fresh agglutinating serum, the former serum contains agglutinoids.

Sometimes it is found that even a fresh serum, when concentrated, will cause less agglutination than when diluted. This has been referred to the presence of agglutinoids which have a stronger affinity for agglutinogen than has the agglutinin; when of this character they are called proagglutinoids, and accordingly are analogous to the protoxoids mentioned earlier. As the serum is diluted the concentration of the proagglutinoids becomes less, and at a time when they are so dilute that they have no influence on the reaction, the agglutinins **Proagglutinoids.**

are still present in such quantity that agglutination is brought about.

Two Stages in Agglutination.

The presence of some salt is necessary for the occurrence of agglutination. Bordet found that if the salts were removed from the serum and from the suspension of bacteria by dialysis, and the two were then mixed, agglutination did not occur; if a small trace of sodium chlorid was added the reaction took place promptly. Furthermore, if the serum was completely removed from the bacteria by repeatedly washing them in distilled water, it was found that the microbes had absorbed the agglutinin, but the latter remained inactive until the salt was added.

This experiment not only suggests a haptophorous as distinguished from a zymotoxic group, but also indicates that agglutination consists of two phases. The first phase represents the union of agglutinin with the bacteria, while in the second are included the other changes necessary for the clumping of the organisms, in which the activity of the zymotoxic group is represented. The action of the salt, just cited, is unknown.

The properties of serums which are of interest in immunity are now being studied by chemists, notably by Arrhenius. The study of mass action, of chemical equilibrium between agglutinin and agglutinogen, for example, and of the dissociation of the compound after it has once formed, are subjects under investigation, but which are too technical to be entered on here.

Group Agglutination.

"Group agglutination" has been referred to. By this is meant the ability of an antimicrobic serum to agglutinate certain other organisms which morphologically, biologically and often pathogeneti-

cally, are closely related to the homologous bacterium. In these instances, the agglutinating power is greatest for the homologous organism, and the degree to which the heterologous organisms are agglutinated is, to some extent, an index of the proximity of the relationship of the latter to the former. Antityphoid serum has been found to agglutinate the psittacosis, colon, paracolon, and paratyphoid bacilli and *Bacillus enteritidis,* but the action is never so strong as on the typhoid bacillus itself. We are to understand that this power to agglutinate related organisms represents something more than the normal property of the serum; there has been an actual increase in agglutinin for the heterologous bacteria as a result of infection or immunization by the primary organism.

Having typhoid fever in mind, this is a rule which works both ways. Infections with the colon bacillus and related organisms, and sometimes with organisms not closely related, as the staphylococcus, may cause an increase in agglutinin for the typhoid bacillus. The importance of this fact is evident, and it may explain the positive Gruber-Widal reaction sometimes found in infections other than typhoid.

Chief Agglutinin and Co-agglutinins.

Inasmuch as the highest agglutinating power is always manifest against the homologous organism, this is spoken of as the chief agglutinin (*Hauptagglutinin*) of the serum, while the weaker agglutinins for other organisms are called partial or adventitious agglutinins, or coagglutinins (*Mitagglutinin*).

Specificity.

The phenomenon of group agglutination would seem to violate the specificity which we are in the

habit of attributing to the reactions of immunity; yet a reasonable explanation has been offered for the occurrence. It is probable that the protoplasms of all cells have certain constituents in common, and that the closer the relationship between two different cells the greater is the similarity of their constituents. In view of this probability, Durham has used the following illustration in the explanation of group agglutinations: The typhoid bacillus contains certain constituents, agglutinogenic molecules, which one may designate as a, b, c, d, and e; these differ among themselves in unknown respects, but each is able to stimulate to the formation of a corresponding agglutinin. The serum, then, would have the agglutinin molecules A, B, C, D and E, also differing among themselves, but having at least one property in common—that of causing agglutination of the typhoid bacillus by uniting with the corresponding agglutinogenic molecules. In this sense nothing could be more specific. The *Bacillus enteritidis*, closely related to the typhoid organism, may possess the agglutinogenic molecules c, d, e, f, g, and h, and following the principle expressed above would stimulate, in the body, to the formation of the agglutinin molecules C, D, E, F, G and H. Inasmuch as the agglutinogens c, d and e are common to the two bacilli, the agglutinins C, D and E, which are present in both serums, would affect either of the two organisms. The typhoid serum, however, would contain five agglutinins for the typhoid bacillus and only three for the *Bacillus enteritidis*, consequently the action would be stronger against the typhoid bacillus; *mutato mutandis*, the same applies to the enteritidis serum.

The same line of reasoning would explain the increased agglutinating power of an anticolon serum for the typhoid bacillus.

A further elaboration of this principle may be made in a case in which two different strains of the same organism (typhoid bacillus) have somewhat different agglutinogenic molecules; consequently the homologous immune serums for the two organisms might not coincide in their agglutinating powers for a third strain of the bacillus.

Importance of Serum Dilutions.

In view of the points mentioned, it is clear that specificity of a given serum may be determined only by diluting the serum to such an extent that the coagglutinins practically are eliminated, the chief agglutinin being present in so much greater concentration that it is still able to agglutinate the homologous bacterium.

Theoretically, it is also important for the specificity of the reaction that the particular strain of the organism to be used for the test correspond in its agglutinogenic molecules or receptors with those of the strain used for the immunization; the agglutinogenic receptors should be typical for the organism.

It is doubtful if group agglutination occurs among all closely related bacteria, inasmuch as Kolle found that it did not exist among the vibrios.

Mixed Infections.

It is thought possible that the multiple agglutinating power of a serum may be caused by mixed infections in some instances. Although this is to be kept in mind, one should not overestimate its diagnostic importance, because a similar multi-

plicity may result from infection by a single micro-organism.

Production of Agglutinins. Ehrlich Theory.

The explanation of the production of agglutinins by the body, according to the conception of Ehrlich, is similar to that already given for the production of antitoxins. That is to say, the agglutinin molecules are cast-off cell receptors, the overproduction of which has occurred as a result of their union with the agglutinogenic molecules of the bacteria. The antitoxin receptors were relatively simple, having no other demonstrable structure than that of the haptophorous groups through which they unite with the corresponding toxin.

Receptors of Second Order.

We have recognized in the agglutinin receptor two groups, a haptophorous and a zymotoxic; consequently it must have this same structure when it is still a part of the cell. Ehrlich designates it as a receptor of the second order, which, being defined, is a receptor in which a haptophorous and a zymotoxic group exist as integral parts of the molecule (Fig. 6).

In accordance with the side-chain theory, the ability of an animal to form agglutinins for a certain organism would depend on its possession of receptors of the second order which are able to unite with the agglutinogenic receptors of the bacterium. It is well established that different animals may not form serums with equal agglutinating powers for an organism. The following is a concrete example: Wassermann immunized rabbits, guinea-pigs and pigeons with a strain of the colon bacillus, and tested the three serums with fifteen other strains of the same organism. The serum of the guinea-pigs readily agglutinated the strain which was used for immunization, but scarcely affected

the others. The serums of the rabbits and pigeons also agglutinated the homologous culture, but the coagglutinins which they possessed did not affect other strains equally. Consequently, it was supposed that the cells of the three animals contained a limited number of receptors in common, whereas

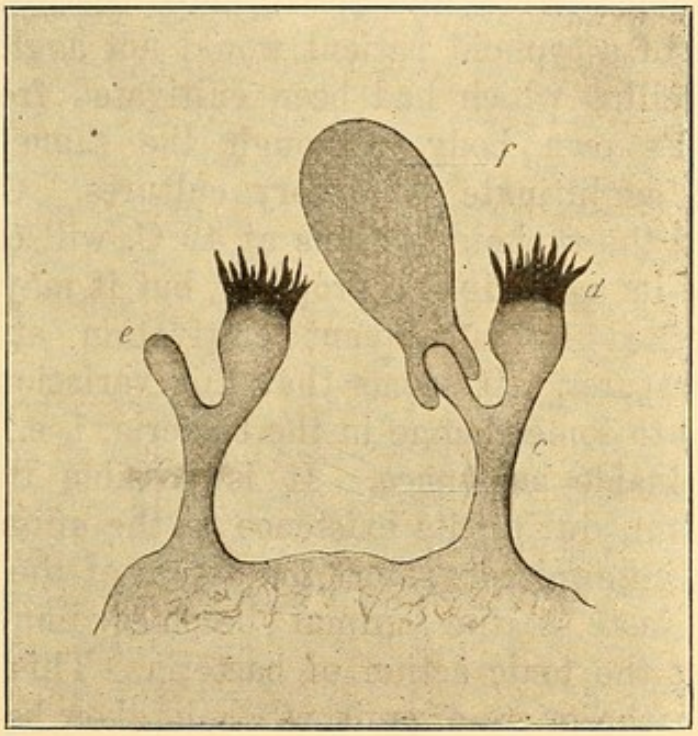


Fig. 6.—Graphic representation of receptors of the second order and of some substance uniting with one of them. *c*, cell receptor of the second order; *d*, toxophore or zymophorous group of the receptor; *e*, haptophore of the receptor; *f*, food substance or product of bacterial disintegration uniting with the haptophore of the cell receptor. From Ehrlich's "Schlussbetrachtungen," Nothnagel's System of Medicine, vol. viii.

other receptors which were present in one of the animals were largely wanting in the other two.

Inagglutinability of Some Organisms.

Inagglutinability was mentioned as a characteristic of certain bacteria, especially the bacillus of Friedlander. This condition is much more important when it involves an organism which

usually is agglutinated with ease. In some instances, the typhoid bacillus when freshly cultivated from a patient, or, indeed, from contaminated water, has been found to resist agglutination by a strong serum; the same organism after a period of existence on artificial media becomes agglutinable. Widal and Sicard noted that often the serum of a typhoid patient would not agglutinate the bacillus which had been cultivated from the patient's own body, although the same serum would agglutinate laboratory cultures. Cultivation of the typhoid bacillus at 42 C. will cause it to lose its agglutinable property, but it may be reestablished by subsequent cultivation at lower temperatures. It seems that this variation must be due to some change in the bacteria, i. e., in the agglutinable substance. It is possible that the organism, during its existence in the animal, becomes immunized against the action of the agglutinin just as the animal becomes immunized against the toxic action of bacteria. This condition in the micro-organisms would then be represented by a great excess of agglutinogenic receptors, so that a much greater amount of agglutinin would be required to cause clumping. It is readily seen how the use of an inagglutinable strain of the typhoid bacillus would affect serum diagnosis.

Theories of Agglutination.

We are to consider that in the phenomenon of agglutination a reaction of a chemical or physicochemical nature takes place between the agglutinin of the serum and the agglutinogen of the microorganisms, the actual clumping following as a consequence of this reaction. It is not a "vital" reaction, for dead bacteria may be agglutinated.

Theories of agglutination have to do, not with the existence of agglutinin and agglutinogen, but rather with the nature of the reaction between the two, and the influences which bring about the clumping after the reaction has occurred. The original theory of Gruber supposed that the serum so affected the bacteria that they became sticky; consequently, as they came in contact, they were, so to say, glued together. Dineur thought changes occurred in the flagellæ of the organisms, a theory which is untenable because some bacteria are agglutinable which do not possess flagellæ. Emmerich and Loew refer agglutination to the action of an enzyme which is produced by the bacterium itself, a theory which is not given general credence. Bordet excludes the vitality or motility of the organisms as factors, and believes that the process is purely a physical one, because of the fact that some known chemical substances may be made to precipitate or to agglutinate certain other substances (precipitation of colloids by salts); the theory presupposes some change in the molecular attraction between the microbes and the surrounding fluid.

Other theories have to do with the question of precipitation. As previously stated, when the filtrates of cultures of certain organisms are mixed with their corresponding immune serums, precipitates occur in the mixtures. It was mentioned that the substance in the filtrate which takes part in the precipitation may represent, in part, the agglutinable substance which has been excreted by the bacteria. Nicolle supposes that the agglutinable substance resides in the external layer of the bacteria and that when the serum is added a coag-

ulation occurs in the envelope, rendering coalescence with the envelopes of other individuals possible. The theory of Paltauf that the agglutinable substance finds its way to the surface of the bacterium and is precipitated by its union with agglutinin is somewhat similar. The shell of the coagulated substance accounts for the sticky character which the envelope acquires, according to the theory of Gruber. Paltauf cites observations which tend to show that some substance actually is extruded from the micro-organisms during agglutination, and that in properly stained specimens it can be seen as a precipitate surrounding and between adjacent organisms.

The multiplicity of theories leads one to suspect that the true nature of the process remains obscure.

CHAPTER XI.

PRECIPITINS.

Because of their scientific importance and certain practical features, the serum-precipitins should receive something more than the incidental mention which has been given them under agglutination and in other chapters.

Bacterial Precipitins.

In 1897 Kraus discovered that bouillon cultures of the organisms of typhoid, cholera and plague, from which the bacteria had been removed by filtration, would cause precipitates when mixed with their respective antiserums. The reaction is specific. As stated later, however, this specificity holds only when those quantitative relationships are observed which were found so essential for the agglutination test. The precipitins of Kraus are the bacterial precipitins. He proposed their use for the identification of micro-organisms. If, for example, one has in hand a culture which he suspects to be that of the typhoid bacillus, it may be grown in a liquid medium, the cells removed by filtration, and the filtrate mixed with a known antityphoid serum; if a precipitate occurs when the serum is sufficiently diluted, the reaction indicates that the organism in question is the typhoid bacillus. Inasmuch as precipitins are formed during the course of some infections it may be possible to use them in clinical diagnosis, but for either bacterial or clinical diagnosis the agglutination test is more readily performed and interpreted.

Phytoprecipitins and Zooprecipitins.

Phytoprecipitins are produced by immunization with albuminous substances of plant origin, as ricin and albumin from grains, and their action is specific for the homologous substance.

Zoöprecipitins are obtained by immunizing with animal albumins. Through the work of Wassermann and Uhlenhuth, of Nuttall, and others, it has been demonstrated as a general law that immunization with an albumin from whatsoever source gives rise to the formation of a precipitin which manifests its action only against the particular albumin used for the immunization. Hence, the albumin of a particular serum, in some unknown respect, is different from that of all others; it is special to the species.

Lactoserum.

Immunization with milk causes the formation of a precipitin which throws down the casein of the milk used for injection, but not that of milk from another species. The milk of the goat may be differentiated from that of the cow by the use of the lactoserum.

Likewise, after the injection of egg-albumin a precipitin is formed which is specific for the type injected.

Precipitogen, Precipitin and Precipitate.

Three substances are open to study in the precipitation reaction. First, the fluid or substance which is used for immunization; it bears the name of precipitogen, i. e., the precipitin-producing substance, Second, the specific constituent of the precipitating serum, i. e., the precipitin. Third, the precipitate, which is a consequence of the reaction between precipitogen and precipitin. We are able to recognize in this instance the actual end-product of a reaction, a condition which is not so easily realized in other "immunity reactions." It

is true, of course, that little has been learned concerning the nature of the end-product; its chemistry is as dark as that of the proteids in general.

Formation of Precipitin.

As stated in the chapter on "Natural Immunity," normal serums occasionally have the power to cause precipitates in other serums. Precipitins for egg albumin and goat serum have been found in extracts of organs, although at the same time they were absent from the serum of the animal. In this case the active bodies exist in the cells as "sessile receptors," and by the process of extraction they are brought into solution. During immunization these same receptors are stimulated to overproduction and are thrown into the circulation as free precipitin receptors.

The power of forming precipitins may be widely distributed among the organs. This function has been assigned to the leucocytes (Kraus and Levaditi, Moll), and in one case they were formed locally in the anterior chamber of the eye (v. Dungern, Römer).

For the artificial production of precipitins the precipitinogenous fluid may be injected into the veins, peritoneal cavity or the subcutaneous tissue. Within from four and a half to five days the precipitin has been formed to such an extent that it may be demonstrated in the serum of the immunized animal.

Concerning Autoprecipitins and Isoprecipitins.

As in the case of agglutinin formation, not all animals have equally the power of forming a precipitin for a given albumin. This point, as related to serum precipitins, is of particular importance, and involves a factor which is of no consequence in bacterial agglutinins. In the first place, an animal will not form a precipitin which

is active against its own serum, i. e., by bleeding an animal and reinjecting the serum a specific precipitin is not formed. If formed it would be an autoprecipitin, and, as a rule, animals do not form antibodies for their own tissue constituents. Again, animals are unlikely to form antibodies for the tissue constituents of other members of the same species; these, when formed, are called isobodies. Schütze immunized thirty-two rabbits with serum from the rabbit and obtained an isoprecipitin from only two of the number. In the third place, animals do not readily form antibodies for the tissue constituents of other animals which zoölogically or biologically are closely related. Immunization of the guinea-pig with the serum of the rabbit, a pigeon with that of a chicken, or a monkey with human serum, are procedures which usually do not yield precipitating serums.

Nature of Precipitins. Chemically, little is known of precipitins. They are thrown down by ammonium sulphate in conjunction with the euglobulin fraction of serum, and are destroyed by those substances which alter albuminous bodies, as acids, alkalies, pepsin and trypsin.

Specific Inhibition. When serum is heated to from 50° to 60° C. its ability to cause a precipitate in the homologous precipitogen is destroyed, although it may be demonstrated that the power to combine with the latter is unchanged. Hence precipitin, like agglutinin, is composed of two groups, a binding or haptophorous, and a ferment-like group in which the active property reside; the latter is the coagulin of the molecule. When precipitin has lost its coagulin it becomes precipitoid, and as precipitoid

it may unite with precipitogen and thereby inhibit the action of a fresh precipitin which may be added later. When a precipitating serum has partly degenerated into precipitoids, that is, when it consists of a mixture of precipitin and precipitoid, it is found that the latter have the greater affinity for precipitogen; hence, in concentrated solutions of the serum, precipitoid may be present in sufficient quantity to bind all the available precipitogen, and the reaction would not occur in spite of the presence of active precipitin. This is spoken of as specific inhibition. The action is analogous to that of toxoids and agglutinoids, and the phenomenon is mentioned again in this instance in order to emphasize the fact that certain principles of action are common to many of the immune substances. Precipitoids, like toxoids and agglutinoids, are formed by long standing, by the action of heat and light and by other injurious influences.

The molecule of precipitin, like that of agglutinin, is a receptor of the second order (Fig. 6).

Antiprecipitins.

The attempt has been made to produce antiprecipitins by immunization with precipitating serums; this is immunization with an immune serum. It is reported that antibodies have been obtained for lactoserum, but not for bacterial precipitins. There is a limit to the cycle of antibody formation.

Nature of Precipitogen.

Precipitogen may be defined as any albuminous substance immunization with which will cause the formation of a specific precipitating serum. In addition to those mentioned above, albuminous urine, pleural exudates, ascitic fluid and that from hydrocele are precipitogens. The same is true of

some albuminous fractions of serums, as globulin, the precipitating serum for which may be called antiglobulin. Kraus believes that the precipitogen of bacterial filtrates is associated with albuminous molecules. Jacoby obtained by tryptic digestion of ricin, a precipitogen which gives no albumin reaction. On the other hand, certain precipitogens are destroyed by pepsin and trypsin, a fact which indicates their albuminous nature.

Certain precipitogens are said to consist of a thermolabile and a thermostabile portion, the differentiation of which we need hardly consider.

Precipitoid Derived from Precipitogen.

It is of no little interest that precipitogen, similar to precipitin, consists of two groups, through one of which it unites with precipitin, whereas the other has a coagulating function. Egg albumin, for example, when heated to rather high temperatures, loses its ability to participate in the precipitation reaction, although it retains its binding power for precipitin. In view of the fact that the two substances which enter into the reaction have similar structures, it is difficult to say which assumes the passive and which the active rôle. Degenerated precipitogen is also called precipitoid. In order to distinguish the two precipitoids one must speak of the precipitoid of precipitogen, and the precipitoid of precipitin. The precipitoid of precipitogen yields precipitin by immunization; hence, it is all the more analogous to the toxoids.

Precipitate.

The precipitate which is caused when a bacterial filtrate is mixed with its specific antiserum forms in from one-half hour to several hours, and appears as a coherent white sediment which in the course of twenty-four hours has left the overlying fluid quite clear. The action of the precipitins for

serums is more rapid, and in either case sedimentation is hastened by placing the fluids at body temperature. As intimated above, the occurrence of the reaction depends on an intact condition of the coagulin groups of both substances. A low concentration of organic acid favors, whereas mineral acids and alkalies inhibit or prevent precipitation; a neutral reaction is indifferent. The precipitate contains albumin, which, however, has become so changed that it is not susceptible to the action of trypsin. The two in combining have in some way shut off the point of attack for trypsin. A lactoserum precipitates the casein of the corresponding milk. The presence of salts is necessary for the reaction of precipitation.

Group Precipitation and Specificity.

Group precipitation is not so pronounced as group agglutination, yet it exists to a certain degree and is of the utmost practical importance in attempting to differentiate serums by the precipitation method. Although bacterial precipitins are highly specific, it is important to observe the principle of serum dilution which was emphasized under agglutination, in order to obtain the adventitious precipitins in such small amounts that they do not interfere with the chief precipitin.

Forensic Use of Precipitins.

That feature of the precipitation reaction which has the most practical bearing has to do with its medicolegal use in the detection of human blood. For this purpose it has supplanted the specific hemolytic serums, which are to be referred to later. In the course of investigations it was found that even the smallest dried blood stain, although months old, would cause the formation of a sediment when mixed with its homologous precipitating serum. It remained for certain important de-

tails to be worked out in order to render the test sufficiently reliable for forensic work. The specificity of the reaction appeared to be threatened somewhat when it was learned that the serum of monkeys undergoes precipitation when treated by an immune serum which is specific for human serum. This is, again, group precipitation. Adventitious precipitation is, in fact, so widespread that some have felt justified in speaking of a mammalian serum reaction. Hence, in order to insure specificity, it has become necessary to use precise quantitative methods in differentiating bloods or serums by this method. The immune serum which is used in the test must be diluted to some extent in order to eliminate accidental precipitins; but even a more important precaution is the volumetric measurement of the precipitate which is formed. The technic of Schur may be cited. Test tubes are so made that the lowermost portion consists of a graduated capillary tube. One c.c. of the fluid to be tested is placed in one of these tubes, to which is then added 0.2 c.c. of the precipitating serum. The mixture is kept at body temperature until the reaction is complete, and the sediment is then thrown into the capillary portion of the tube by centrifugation for a stated period of time (twenty minutes). The volume of the sediment may be read by the scale. Nuttall allows the sedimentation to occur naturally, with the tubes in an upright position. Other serums naturally must be used as controls. If the "unknown" blood is suspected of being human, a control tube must be prepared in which a similar amount of known human serum is submitted to the same test. If the two tubes yield similar amounts of precipitate

when they are treated with 0.2 c.c. of a precipitin which is specific for human serum, the identity of the "unknown" blood as that of man is established. To obtain the specific precipitin it is customary to immunize rabbits with human serum for several weeks.

Identification of Meats.

Another practical feature of the precipitation test has to do with the differentiation of meats. A precipitogenous substance which is characteristic for the animal may be extracted or pressed from the flesh, and will yield a precipitate when it is mixed with a precipitin of homologous nature. This is of particular interest in those countries in which the meat of the horse is put on the market as a substitute for that of beef.

The possible relationship of precipitation to bacterial agglutination was referred to in the chapter on agglutination.

Colloids and the Reactions of Immunity.

In view of the fact that the protoplasm of the body and the albuminous constituents of serum have a close relationship to, or really are, colloids, investigators have studied certain reactions which occur among the known colloids with the expectation that the reactions of protoplasm and those of serums would receive some elucidation. Not much advancement can be made, however, until the properties of colloids are more thoroughly understood.

Substances which go into solution were classified by the English physicist, Graham, as crystalloids and colloids. Crystalloids include many inorganic salts. Usually they form clear solutions in water and exert osmotic pressure, supposedly because of the small size of their molecules. They diffuse with some rapidity and many are conductors of electricity. Organic colloids comprise such

Properties of Colloids.

substances as albumin, starch, dextrin, tannin, gelatin and many gums. By proper treatment of certain metals and their salts, inorganic colloids may be prepared; for example, ferric hydroxid and the sulphids of antimony and arsenic. When colloids are dissolved in water the solutions are often more or less opaque, and are sometimes opalescent because the particles or molecules are of such size that they polarize light. They exist in water either as a solution of molecules of great size or as a suspension of considerable particles or aggregates of molecules. In some instances the particles are so large that they may be seen by a magnification of 1,000 diameters, while in others no degree of magnification renders them visible with the ordinary microscope. By the use of the recently devised ultramicroscope, however, the finest particles in some colloidal solutions may be discerned. Colloidal substances, such as albumin, diffuse very slowly and exert little or no osmotic pressure, supposedly because of the large size of the particles. They do not conduct electricity, but the particles themselves react to the electric current by alterations in the direction of their motion (i. e., toward the positive or the negative pole), and, moreover, carry electric charges themselves.

Precipitation of Colloids by Electrolytes.

The features of colloids which bring them into relation with the subject in hand are their coagulable nature in certain instances and the fact that their particles may be agglutinated or precipitated by the addition of minute amounts of salts (electrolytes). In this connection one naturally recurs to the observation of Bordet, which was mentioned in the preceding chapter, concerning the inagglutinability of micro-organisms so long

as salt is withheld from the solution. This analogy would suggest that the bacteria after their union with agglutinin may conduct themselves as colloidal particles. In the precipitation of colloids by salts it has been suggested that the salts so alter the electric condition of the colloidal particles that their surface tension is decreased, and as a result of this change neighboring particles coalesce in such quantities as to produce a visible sediment.

Neisser and Friedberger have studied certain colloids, having in mind the similarity of their behavior to serum reactions. They found, for example, that two of our common dyes which are colloids and bear opposite charges of electricity (eosin and Bismarck brown), give rise to a precipitate when the two are mixed. Furthermore, the specific inhibition which may be obtained in the reaction with serum precipitins (see above) could also be realized with the eosin and Bismarck brown.

The agglutination of bacteria and of red blood cells may also be accomplished with colloids. Landsteiner agglutinated erythrocytes with colloidal silicic acid.

CHAPTER XII.

A. GENERAL PROPERTIES OF BACTERICIDAL SERUMS.

Bacteriolysis and Bacteriolysin.

Antibacterial, bactericidal and bacteriolytic are three terms which are used in a rather loose, interchangeable way, although they are not strictly synonymous. A bactericidal serum is one which is able to kill bacteria, as the term implies; if at the same time it dissolves the organisms it is bacteriolytic. Inasmuch as some serums do kill bacteria without dissolving them (typhoid), while others have the dissolving power (cholera), the distinction has a certain significance. In either case the serum is, of course, antibacterial. For lack of a more concise English term, bacteriolysis is used to designate the process in which bacteria, with or without solution, are killed by serums. Bacteriolysin refers to the substances in serum which accomplish this action. The means of determining the bactericidal power of a serum were indicated in Chapter V, C. True bacteriolysis is best observed with the organism of cholera and its antiserum as described later under the title of the Pfeiffer experiment.

Bacteriolysins are far more complex than antitoxins, agglutinins and precipitins. One may best appreciate their nature as understood at present by tracing their development from the relatively simple alexins of Buchner.

Alexins.

Following the investigations of Fodor, Behring and others, which showed that normal blood may kill bacteria in the test-tube, and after additional

facts were obtained by Nuttall, Buchner demonstrated that it is not necessary to use the full blood in order to obtain the bactericidal action, but that serum alone has a similar effect. He spoke of the antibacterial substances collectively as alexins (substances which ward off), taking the reasonable view that natural immunity to bacteria depends on their presence in the body. The increased bactericidal power of the serum which develops during immunization or infection with certain micro-organisms goes hand in hand with the increased resistance of the individual against the infection. The alexins have undergone a specific increase; they are now immune alexins or, as we say to-day, immune bacteriolysins, and it is supposed that acquired immunity, in these instances, depends on their new formation.

Selective Action.

Alexins were very sensitive substances; they disappeared spontaneously from serums in a few days, were destroyed by a rather low degree of heat (55° C.), by acids and alkalies, and were active only in the presence of certain salts, especially sodium chlorid. A striking feature of alexins, as distinguished from chemical bactericides, was their marked selective action on bacteria. The alexins of animal A might destroy one micro-organism readily and affect another little or none at all, whereas those of animal B might have different selective characteristics.

The Phenomenon of Pfeiffer.

Work which was instituted by Pfeiffer and developed further by others led the way to a more correct understanding of the nature of alexins. Pfeiffer studied the bactericidal action of serums in the body of the living animal, i. e., in the peritoneal cavity. His most classic results were ob-

tained with the organism of cholera. A guinea-pig is immunized against this microbe by injections of the killed or living organisms. We have already learned of this process as that of active antibacterial immunization. When the animal is well immunized the experiment is begun by the intraperitoneal injection of a quantity of culture which would be fatal to an unimmunized animal. At intervals during the next twenty or thirty minutes small amounts of peritoneal fluid are removed for microscopic examination by means of fine pipettes which have been drawn out in the flame. The abdominal wall is punctured with the pipette through an incision in the skin and the fluid flows into the tube by capillary attraction. A portion of the fluid is examined as a hanging-drop or dried on a cover-glass, fixed in the flame and stained with a dilute solution of carbol-fuchsin. In the hanging-drop it is first noticed that the organisms have lost their motility; the comma- and S-shaped forms soon become spherical and at first appear swollen and clear, whereas in later preparations they gradually decrease in size and show a very rapid vibrating movement, the so-called Brownian movement, which is purely physical in nature. In the course of from twenty to thirty minutes the organisms have been completely dissolved. These changes may be followed in the stained specimens, in which the altered cells eventually appear as fine red granules.

The Experiment in Vitro.

As Metchnikoff, Bordet and others have shown, the same result may be obtained without the intervention of the animal body, by mixing perfectly fresh anticholera serum with the vibrios and mounting as a hanging-drop preparation. The

slide must be kept at the temperature of the body by means of a warm stage. The reaction, however, is far less vigorous than when it takes place in the peritoneal cavity and the solution of the cells may not be complete. No bacterium is so completely dissolved under these conditions as the vibrio of cholera, although the typhoid bacillus and similar organisms undergo some changes in their form.

The Activation of an Inactive Serum by the Tissues.

The experiment of Pfeiffer may also be conducted in the abdominal cavity of a non-immune guinea-pig by injecting anticholera serum in conjunction with the culture (passive antibacterial immunization). This is the classic Pfeiffer experiment. The immune serum should be of such strength and should be given in such quantity that the animal is saved in spite of the ten fatal doses of culture which the typical experiment demands. Experiments brought to light a condition which seemed paradoxic; an old immune serum which had lost its bactericidal power as manifested *in vitro,* or one in which the alexins had been destroyed by a temperature of 60° C., showed its original protective power in the animal experiment. Furthermore, when an inactive immune serum was injected into the abdominal cavity, allowed to remain for a time and then withdrawn, its bactericidal power for experiments *in vitro* was found to be re-established. On the basis of these facts, Pfeiffer concluded that the specific substance is present in the immune serum in an inactive form, and that it becomes active as a result of contact with living tissue cells, supposedly the endothelial cells of the peritoneum. According to this conclusion, an inactive serum could become

active again only after its introduction into the body.

Inactivation and Reactivation.

It remained for Bordet to show, on the contrary, that contact of the serum with living cells was not necessary to render it active for bactericidal experiments *in vitro*. It was sufficient to add to the heated immune serum a small amount of fresh normal serum from some normal animal, the quantity of normal serum which was used not being in itself bactericidal. Under these conditions, then, we have to do with two serums which, when combined, are bactericidal, but when separated are inactive. The destruction of the active property of a serum by heat or by other means is called inactivation, and the re-establishment of its power by the addition of fresh normal serum is reactivation. The immune serum, when heated to 55 to 60° C., loses something which is essential to its activity, and this something may be replaced by the normal serum. That the substance in the normal serum is identical with that which was destroyed in the immune serum is indicated by the fact that it is destroyed by the same degree of heat; a heated normal serum will not reactivate an immune serum.

Two Substances in a Bactericidal Serum.

The conclusion of Bordet that the bactericidal power of a serum depends on the combined action of two substances has been substantiated by numerous investigators. These are the substances which in recent years have become familiar under the names of amboceptor and complement and their various synonyms (see p. 141ff). One of them, the amboceptor, is heat-resistant (thermostabile), i. e., it is not destroyed at 56° C., whereas the other, the complement, is susceptible to heat

(thermolabile), being destroyed at that temperature which killed the alexins of Buchner. The term alexin is still applied by some writers to the thermolabile substance (complement), its original significance having been modified.

Specificity.

The specificity which prevails among antitoxins and agglutinins is found also in the action of bactericidal serums. When an anticholera serum is injected into the peritoneal cavity of a guinea-pig, protection is not afforded against other vibrios or other pathogenic organisms. The specificity is so great that the reaction of Pfeiffer may be used for the identification of bacteria. If one has in hand an unknown vibrio, its identity or non-identity as the organism of cholera may be determined by injecting it, in conjunction with anticholera serum, into the peritoneal cavity of a normal guinea-pig; if the microbe is transformed into granules it is the vibrio of cholera, otherwise it is not. Other bacteria may be identified in a similar manner by the use of the proper serums. In spite of this high specificity, the group reaction may occur even with bactericidal serums. An anti-typhoid serum, for example, shows its strongest bactericidal power for the typhoid bacillus, although it is at the same time more destructive for closely related organisms, as the colon bacillus, than a normal serum from the same species. By diluting the serum sufficiently the adventitious bacteriolysins are so nearly eliminated that the specificity of the serum for its homologous organism becomes manifest.

Group Reaction.

The Bactericidal Power in Relation to Immunity.

Bactericidal serums are not obtained with equal readiness for all micro-organisms. We are most familiar with those which are yielded by immuni-

zation or infection with the microbes of cholera, typhoid, plague, the colon bacillus and related bacteria. Many other bacteria, as the pneumococcus, streptococcus, tubercle bacillus and others, yield neither antitoxins nor bactericidal substances. Inasmuch as recovery from such infections is an expression of acquired immunity, no matter how temporary it may be, it is evident that not all examples of acquired immunity can be explained on the basis of the serum properties which we now recognize (see Chapter V, C). This will be referred to again in relation to phagocytosis (Chapter XIV).

Experiments of some importance have to do with the ability of bacteria to absorb the homologous bactericidal substance from a serum when the two are mixed in test-tubes. Hence, if natural antibacterial immunity depends on the bacteriolysin which is present in the circulation, a large mass of the bacterium when injected intravenously should absorb or fix the bactericidal substances; as a consequence, serum which is drawn later should show a great decrease in its bactericidal power for the organism which was injected. Although results of this nature have been obtained by a number of competent investigators, they are not without exception. In the same connection fatal infections should be accompanied by a decrease of the natural bactericidal power of the serum for the organism involved. This has been found to be true in man in relation to plague, and in some animal infections.

The Effect of Bactericidal Serums on Endotoxins.

In a preceding chapter micro-organisms were divided, first, into those which secrete soluble toxins, immunization with which causes the forma-

tion of antitoxins, and, second, those which do not secrete such toxins and for which no manipulations known at the present time are successful in stimulating to the formation of antitoxins. These lines, however, can not be drawn sharply, for there are a few microbes which, according to manipulation, cause the formation of either an antitoxic serum or a bactericidal serum. In general it may be said that the character of the serum depends on the bacterial constituent which is used for immunization. If the diphtheria bacillus itself, or the pyocyaneus bacillus, is injected, the toxin having been washed away, bactericidal serums are formed, whereas if toxins alone are introduced, antitoxins are the result. After all, it seems plain that the bacteria of the second group must be pathogenic, because of toxic substances which they carry with them into the body. In view of the fact, however, that they do not secrete soluble toxins in culture media, it is held that their toxic properties are integrally associated with the bacterial protoplasm; they are the endotoxins spoken of previously.

The question naturally arises: Does a bactericidal serum in dissolving or killing its homologous organism neutralize the endotoxin at the same time? On the basis of very positive experiments which have been performed, especially by Pfeiffer, it is evident that the serum has no such action. In the experiment of Pfeiffer, one may inject into the abdomen a sufficient quantity of anticholera serum to kill all the organisms which have been introduced, and yet the animal may die with the intoxition of cholera. Furthermore, if one considers a culture of the cholera vibrio, which has been killed by heat, as representing so much cholera toxin,

anticholera serum protects against no more of it than does the same quantity of normal serum. It is believed that anticholera and similar immune serums may even increase intoxication by dissolving the bacteria and thus liberating an excess of endotoxin.

Origin of Bactericidal Substances.

We have little positive knowledge concerning the organs which form the bactericidal substances in acquired immunity. Pfeiffer and Marx, in relation to cholera, and Wassermann in typhoid, found that the spleen and the hemopoietic organs in general contain the immune bodies in greater concentration than the blood serum, and in immunization experiments the bodies may be demonstrated in these organs at a time when they are absent from the circulation. This fact is generally accepted as proof of their formation at these points. Wassermann and others have demonstrated the presence of complement in the leucocytes, and Metchnikoff holds that it is produced only by such cells.

Standardization.

The standardization of bactericidal serums is at present more of theoretical than of practical interest, because of their limited therapeutic use. Their values can not be determined with the accuracy with which one measures a unit of antitoxin. One may deliver from a pipette a definite quantity of toxin and if the toxin has been well preserved the same quantity may be obtained at any subsequent time. On the other hand, it is impossible to preserve a culture of living bacteria so that the number of the organisms and the virulence of the culture remain constant, nor will two cultures made at different times contain the same number of cells in a given volume. Hence, standard cultures which are necessary for the systematic

valuation of serums are not easily available. One may use a definite volume of a bouillon culture of an organism which has grown for a certain number of hours, but in all likelihood no two cultures would contain the same number of organisms. Pfeiffer uses the normal loop which has been mentioned, i. e., one which will take up from a surface of agar two milligrams of the bacterial mass. The culture must have grown for a definite period, eighteen to twenty-four hours. Tests having some value may be made in the test-tube with the fresh or complemented serum. This, however, gives one only the bactericidal power as it is manifested outside the body, and it may not be a correct index of the protective power of the serum when it is injected into the living animal. For the test-tube experiment various dilutions of the serum are made, as 1 to 10, 1 to 100 and 1 to 1,000, and a similar quantity of each dilution, properly complemented, is mixed with a given mass of the culture; the mixtures are then placed in the thermostat for a number of hours. At the end of this time plate cultures are made from each of the mixtures, the plates put aside for twenty-four hours, and the colonies which have developed are then counted. The quantity of serum required to kill all the bacteria may be taken as the basis for computing its bactericidal value.

When the protective power of the serum is determined by animal experiment it is not essential to use the serum when fresh; in fact, the native complement in the immune serum may be disregarded, or, preferably, it may be destroyed by heat. If the latter procedure is adopted, or if an old serum is used in which the complement has de-

generated, its reactivation is accomplished through the complement which is present in the body of the experiment animal. It will appear in more detail in the following pages that a given antiserum requires a particular complement for its reactivation, and that this complement may be present in some animals and absent in others.

To find the value of anticholera serum Pfeiffer prepares dilutions similar to those mentioned above, and to the same quantity of each dilution adds ten fatal doses of a virulent culture of the vibrio of cholera. These are injected into the peritoneal cavities of guinea-pigs and after periods of forty to sixty minutes hanging-drop preparations are made from the peritoneal fluid of each animal to determine the formation of the characteristic granules; the highest dilution which causes this change in the cells stamps the value of the serum. The animal must at the same time be protected against the ten fatal doses of the culture.

The value of an antityphoid serum may be determined in the same way, the result being judged by the protection which is afforded the animal rather than by the formation of granules.

Antityphoid, antiplague, and some other serums are also tested by injecting the serum twenty-four hours in advance of the culture.

It is necessary to know the virulence of a culture with which an antiserum is tested. It is possible to maintain some organisms at a rather constant virulence by passage, i. e., infecting animals with the microbe and recultivating it from the tissues. With others, abundant controls must be made at the time the serum is tested in order to know at that moment the precise virulence of the

culture. In all probability it requires more serum to protect against very virulent cultures than against those of less virulence.

Non-Specific Increase in Resistance.

In contrast to the specific immunization which may be accomplished with an immune serum, it is important to recognize that a non-specific increase in resistance may be caused by the injection of a number of substances, which in the test-tube have no destructive action on the bacteria. Issaeff injected into the peritoneal cavity such substances as bouillon, tuberculin and sterile urine, and found the resistance of the animals increased to peritoneal inoculation of virulent organisms. Normal serum from another animal has a similar effect, but, in this instance, the bactericidal substances of the foreign serum may be a factor in the new resistance. Supposedly, this non-specific resistance is local, and it appears to depend on the attraction of an increased number of phagocytes and of additional complement to the peritoneal cavity. The suggestion recently made that preceding laparotomy nucleinic acid be injected into the abdominal cavity, in order to increase the local resistance, has its foundation in the experimental work just cited.

B. AMBOCEPTORS AND COMPLEMENTS.

Experimental Value of Hemolysins.

The simplicity of hemolytic experiments and the rapidity with which they may be performed and terminated have rendered hemolytic serums particularly useful in the study of amboceptors and of complements, for we are to understand that such serums are toxic to erythrocytes only because of the amboceptors and complements which they contain. The most important facts which have been learned concerning the action of hemolytic serums

have been found to hold true for bactericidal serums as well; hence it is an indifferent matter if principles which are common to both are illustrated by frequent references to serum-hemolysins.

Technic of Hemolytic Experiment.

The corpuscles for hemolytic experiments are obtained by the defibrination of freshly-drawn blood and the removal of the fibrin. Usually they are made into a 5 per cent. suspension by dilution with isotonic (physiologic) salt solution. Inasmuch as the serum which is present may interfere with the action of the complement or amboceptors of the hemolysin, it is customary to remove it by a washing process. The 5 per cent. emulsion, or the undiluted blood is centrifugated, the overlying fluid drawn off by means of a pipette and substituted by fresh salt solution; the corpuscles are thoroughly mixed with the new solution and the process of centrifugation repeated, the corpuscles finally being diluted to the original volume with salt solution. After from two to four washings any residual serum usually may be disregarded. To test the hemolytic power of a serum one measures identical quantities of the 5 per cent. washed blood into each of a series of test-tubes by means of a graduated pipette and then adds increasing quantities of the serum to succeeding tubes. All tubes are then diluted to equal volumes by means of salt solution, as it is of some importance to maintain a uniform concentration of the corpuscles. The contents of the tubes are mixed evenly by shaking and the series is placed in the thermostat for about two hours; this temperature is necessary for complete and rapid action of the toxic substances. At the end of this time the tubes are placed in the ice chest and left over night in

order that the cells may settle to the bottom, or sedimentation may be accomplished at once by centrifugation.

Hemolysis.

In either case, the overlying fluid is colored red by the dissolved hemoglobin in proportion to the extent of destruction of the erythrocytes. In case solution has been complete, the sediment is indistinct and colorless, being made up only of the stromata of cells, whereas in the tubes showing only partial hemolysis the sediment is red and has an indirect quantitative ratio to the coloration of the overlying fluid. By suitable variations in the amounts of serum used in different tubes, its exact dissolving dose for the given volume of corpuscles may be determined. Although the term hemolysis is a perfectly proper one, we are to understand that serums cause solution of the hemoglobin, but not solution of the whole cell; we speak loosely of solution of the corpuscles.

Similarity Between Bactericidal and Hemolytic Action.

After Bordet had shown the analogy between bactericidal and hemolytic serums, and after the phenomena of inactivation and reactivation had been developed by Bordet and Metchnikoff, Ehrlich and Morgenroth undertook the study of amboceptors and complements as they occur in hemolytic serums. The facts ascertained by them and the methods of research which they devised have provided many investigators with a starting point for work of the highest importance concerning the bactericidal serums and antibacterial immunity, and their interpretations, moreover, served to extend the side-chain theory of immunity to its present comprehensive limits.

For the sake of convenience one may speak of a heated immune serum, or one in which the com-

Solutions of Amboceptors and Complements.

plement has become inactive from age, as a solution of amboceptors, disregarding temporarily the agglutinins, precipitins and perhaps other bodies which the serum contains. Also, since fresh normal serums are rich in complements and usually contain but a small amount of any one amboceptor, they may conveniently be considered as solutions of complements; yet normal serums may not be considered as pure complement and used as such in unlimited quantities for actual experiments, because of the bacteriolysins and hemolysins which many contain. Only a quantity of the normal serum which in itself is not toxic for the cell may be used for complementing purposes, and this may be as low as, or lower than, 0.1 c.c. for a particular experiment.

The Absorption of Amboceptors by Cells.

As pointed out in the preceding chapter, the combined action of amboceptor and complement is necessary for the cytotoxic action of a serum. In view of the fact that the toxic power is lost by exposure to that temperature which destroys complement, it seems that the latter is the actual dissolving or toxic substance, whereas the amboceptor must play some intermediary rôle. Investigations have shown that the two act together in a very definite manner in that the absorption of the amboceptors by the cells is a prerequisite for the absorption and action of the complement. This may be verified by simple experiments. Mix erythrocytes with the homologous amboceptors, and after a period of from twenty to thirty minutes centrifugate the mixture and remove all the free serum from the cells by repeated washings with isotonic salt solution. If the cells are again suspended in salt solution and a small amount of

complement is added and thoroughly mixed, the hemoglobin is dissolved out; a control must, of course, show that the complement alone has not the dissolving power. The result indicates that the erythrocytes during their contact with the immune serum had absorbed or combined chemically with the amboceptors, and that the latter remained attached to the cells in spite of the washings to which they were submitted.

Sensitization

It would seem that the union of amboceptor with cell has the effect of rendering the latter susceptible to the action of complement, and for this reason amboceptor-laden cells are spoken of as sensitized cells. Hence, according to the cells and serums employed, we may refer to sensitized erythrocytes, sensitized bacteria, etc. The experiment is called the sensitizing, absorption or binding experiment. An immune serum may be deprived of all its amboceptors in the binding experiment if a sufficient quantity of cells has been used, and it would thereby be rendered incapable for further reactivation by the subsequent addition of complement.

Order of Action of Amboceptor and Complement.

If, instead of performing the experiment in the manner described, the process is reversed so that the corpuscles are first treated with the solution of complement and then with the amboceptors, the corpuscles are not hemolyzed. During the washing process the complement is entirely separated from the cells, and from this fact it is clear that direct union between corpuscle and complement does not occur; only sensitized cells take up complement.

The question as to whether the corpuscles in taking up amboceptors do so by chemical combina-

Cytophilous Haptophore of the Amboceptor.

tion or by physical absorption has been contended with some vigor. Ehrlich believes that the process is one of chemical union, and if one adheres to this view it becomes necessary to assign binding or haptophorous groups both to the red blood cells and to the amboceptors. In contrast to another haptophore which the amboceptor possesses and which will be described below, that one which unites with the cell is called the cytophilous haptophore. The haptophore of the erythrocyte which enters into the union is an essential part of a receptor of the red cell, consequently we say that the amboceptor unites with a receptor of the corpuscle.

The Absorption Experiment in the Cold.

The heating of serum to 56° C. provides one means of apparent isolation of the amboceptor from the complement, but this is not a true isolation in that complement is merely rendered inactive by the heat rather than totally eliminated.

Ehrlich and Morgenroth devised a method by which the amboceptors may be separated from a fresh immune serum without in any way injuring the complement. This is accomplished by performing the binding experiment, already alluded to, at a low temperature. The serum, containing both amboceptors and complement, is cooled to 0° C. or slightly above, by means of a freezing mixture of salt and ice. A suspension of the homologous corpuscles is cooled to the same point, the serum is added and the mixture maintained at 0° to 4° C. for fifteen to twenty minutes. At the end of this time the sensitized cells are removed by immediate centrifugation at a low temperature, and are washed entirely free from serum by the use of ice-cold salt solution. If the low temperature has been adhered to rigorously and the work

done quickly, the corpuscles are not laked during the manipulations in spite of the presence of both amboceptors and complement. Furthermore, the washed sensitized cells remain intact even when their temperature reaches that of the thermostat, whereas if some fresh normal serum or the serum from which the amboceptors were absorbed is added, they undergo hemolysis as readily as when treated with the active immune serum. The original immune serum is now a solution of complement, and fresh corpuscles which are added to it are not dissolved because of the absence of amboceptors.

These results show the following important facts: Amboceptor and complement exist side by side in an immune serum, not as a united substance. Union of amboceptor with cell is independent of complement, the latter being taken up only after the amboceptor-cell reaction has occurred. Amboceptors unite with cells at a low temperature, whereas complement requires a higher temperature for its union and for the ferment-like activity by which it dissolves or kills the cells.

Complementophilous Haptophore of Amboceptor.

That constituent of the amboceptor which unites with the cell has been referred to as the cytophilous haptophore. Ehrlich and his followers believe that complement in establishing connection with the cells does so by combining with a second haptophore of the amboceptor, after the latter has sensitized the erythrocyte or bacterium. Hence, an amboceptor has, as the name implies, two receiving groups or haptophores, the second being the complementophilous haptophore (Fig. 7). It is hardly desirable to discuss various ex-

periments which furnish additional evidence of the amboceptor nature of the thermostabile body. The observed phenomena allow one to assign to it the two haptophores mentioned.

Action of Amboceptors.

There is a conflict of ideas as to the nature of the change produced by the amboceptors, as a result of which the cells are made susceptible to the action of complement. Bordet speaks of the amboceptor as the *substance sensibilisatrice,* the sensitizing substance; and his conception of the action of the two substances he has compared roughly to the opening of a lock for which two keys are demanded. One key, amboceptor, is needed to prepare the lock for the action of the second key, complement, the latter being the one which really opens it.

Metchnikoff applies the name fixator to the thermostabile body, having in mind the action of a mordant in preparing tissues or other substances for the reception of a dye; this differs little from preparator, the word used by Gruber.

The idea of Ehrlich, however, is distinctly at variance with the conceptions mentioned, for he sees in the union of amboceptor with cell nothing more than the introduction of a new chemical affinity, i. e., one which attracts complement, and this new affinity does not lie in the cell itself, but rather in the amboceptor (complementophilous haptophore) after the union has occurred. Hence, the terms intermediary body (*Zwischenkörper*), copula of Müller, and desmon of London, are words which carry with them the meaning that the amboceptor first unites with the cell and then acts as a linking substance through which complement finally is put in relation to the cell. This also is

the meaning embodied in the amboceptor of Ehrlich, the word indicating more accurately his conception of the method by which the substance acts as an intermediary body.

Structure of Complement.

If we consider it established that in the process of cytolysis union occurs between complement and amboceptor we must at the same time assign a haptophorous group to complement. Union would be impossible without it. Corroborative proof of the existence of this haptophore lies in the fact that immunization with complement results in the formation of anticomplement, a prerequisite for which is union of complement with cell receptors in the immunized animal; and this union, it seems necessary to assume, takes place through a binding group. The mere possession of a haptophore, however, does not account for the ferment-like activity of complement. The latter characteristic resides in the so-called zymotoxic group; hence, complement, having a binding and a toxic group, has a structure like that of a toxin.

Complementoid.

Somewhat loosely we have said that the inactivity of a serum which has been heated to 56° C. depends on destruction of the complement. This is not strictly true, however, for such treatment destroys only the zymotoxic group, the haptophorous constituent remaining uninjured. Complement altered in this respect is called complementoid, and it is analogous to toxoid, agglutinoid and precipitoid. Two essential facts go to show that this is the principle change wrought by heating. First, the fact stated above, that immunization with complementoid, causes the formation of anticomplement. Second, complementoid may exceed true complement in its affinity for the

amboceptor, and if sensitized cells are treated with a serum containing a mixture of complement and complementoid, the latter may occupy completely the complementophilous haptophores of the amboceptors and thus may block the way for action on the part of complement. This is again the specific inhibition which has been mentioned in connection with toxoids, agglutinoids and precipitoids. This is the *Complementoid-Verstöpfung* of Ehrlich.

Formation of Amboceptors.

The amboceptor, as the characteristic property of a bactericidal or of a hemolytic serum, is a specific product of the immunization, whereas the amount and character of complement in the immunized animal undergoes little or no change. We are, of course, obliged to consider the amboceptors as a product of the cells of the body. In the terminology of Ehrlich, they are discarded cell receptors, and with their two haptophores represent a more complex structure than either the receptors of antitoxin or agglutinin; the latter are uniceptors; the former amboceptors, and because of their higher differentiation Ehrlich has called them receptors of the third order (Fig. 7).

When micro-organisms gain entrance to the body they are killed and dissolved in considerable masses. As a result of the solution, certain bacterial constituents reach the circulation, and among them are molecules or receptors which possess haptophores capable of uniting with a particular type of amboceptor, the latter being an integral part of some tissue cells. This union having taken place, an affinity for circulating complement may be created as in the test-tube experiments. We have thus the possibility of stimula-

tion of the cell by the bacterial constituent itself as a toxic or unusual food substance, or the toxic action may be caused by products of disintegration of the bacterial substance, the disintegration having been accomplished by the digestive action

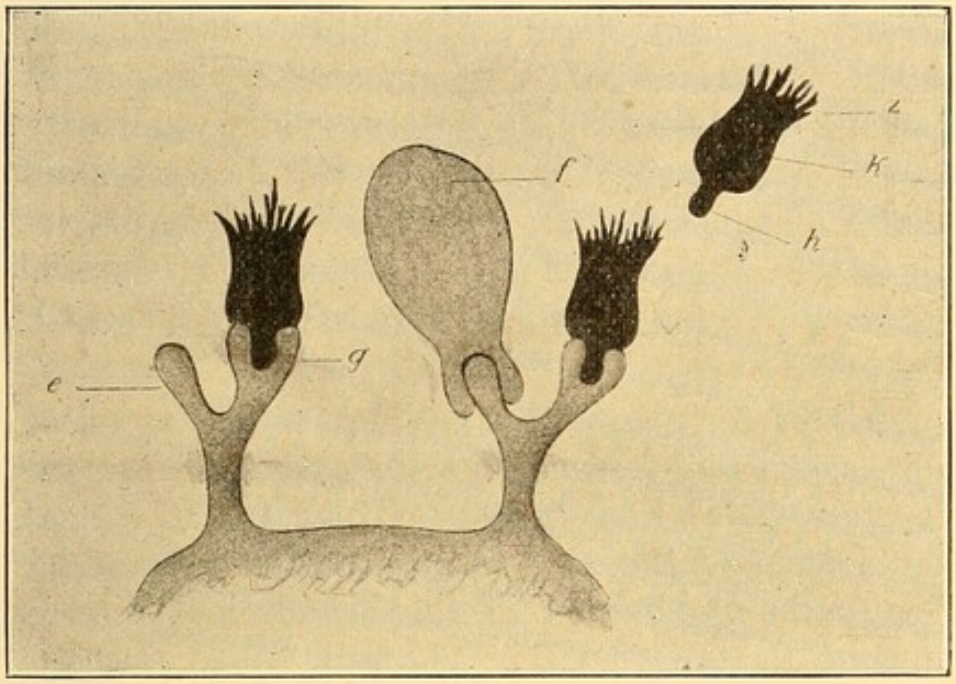


Fig. 7.—Graphic representation of receptors of the third order, and of some substance uniting with one of them. *c*, Cell receptor the third order, an amboceptor; *e*, one of the haptophores of the amboceptor, with which some food substance or product of bacterial disintegration, *f*, may unite; *g*, the other haptophore of the amboceptor with which complement may unite; *K*, complement; *h*, the haptophore, and *z*, the zymotoxic group of complement. From Ehrlich's "Schlussbetrachtungen," Nothnagel's System of Medicine, vol. viii.

of the complement which was taken up by the amboceptor. The effect is that of an unusual stimulation, in response to which the cell, if not fatally injured, reproduces many amboceptors corresponding to the type which was occupied or injured. As in the formation of other antibodies, the new-

formed amboceptors reach the general fluids of the body.

Specificity of Bactericidal Amboceptors and Complements.

Concerning the specificity of serum-hemolysins and serum-bacteriolysins for their homologous cells, we, of course, refer to the specificity of the whole amboceptor-complement complex. It is necessary to throw the responsibility on both substances, because of the variations which exist among complements as well as among amboceptors. Inasmuch, however, as the heat-resistant body alone is increased during immunization or infection, the greater part of the specificity would seem to depend on the nature of the amboceptor rather than on that of complement.

Bacterial Receptors.

All bacteria which stimulate to the formation of bactericidal serums do so because of certain receptors which they possess. These are, of course, analogous to the receptors of erythrocytes which cause the production of the hemolytic bodies in serum. Bacteria have, in addition, many other receptors, some of which cause the development of agglutinins. In the latter instance we speak of the agglutinogenic receptors of the cells, but there is no name of equal convenience which is used to designate the receptors which stimulate to the formation of amboceptors. No two micro-organisms contain an identical receptor apparatus; if the contrary were the case their antiserums would coincide in their bactericidal action. Therefore, the cell receptors (amboceptors) with which they unite during immunization differ correspondingly in their cytophilous haptophores. The cytophilous haptophore of the typhoid amboceptor finds its specific counterpart in the typhoid bacillus, and finding no such counterpart in the vibrio of chol-

era, the latter can not be sensitized by the antityphoid serum; on this fact depends the specificity of the serum. This conception does not interfere with the explanation of the group reaction among bactericidal serums, for it is conceivable that the colon bacillus, for example, has, in addition to those receptors which characterize the organism, a small percentage of receptors which are identical with those characterizing the typhoid bacillus. In accordance with this possibility an antityphoid serum may well, as it does, show some increased bactericidal power for closely related organisms. Hence the explanation of group bacteriolysis is identical with that of group agglutination.

Multiplicity of Complements.

There is a wide difference of opinion regarding the unity of complement, or alexin, its synonym. Bordet and his followers stand for the unity of the alexins, and their position rests on the fact that a given normal serum may be used to activate many different amboceptors. We should appreciate that this phenomenon might depend on the broad range of action of a single complement, or on the presence of different complements each being specific for a particular amboceptor. Ehrlich and his school take the latter view and have actually demonstrated a multiplicity of complements in a few instances. Ehrlich and Sachs treated fresh normal serums (complement) in various ways, such as digestion with papain, partial destruction with alkalies, heat, etc., and were able by these methods to destroy the complement for one kind of amboceptor, while the serum still retained its power for activating other amboceptors. Accordingly, it seems clear that the ability of a normal serum to activate a given amboceptor depends not only on

the presence of complement in a general sense, but on the presence of a suitable complement, i. e., one the haptophore of which corresponds to the complementophilous haptophore of the amboceptor. This point is of great importance in reference to the treatment of infectious diseases with antibacterial serums, for the efficacy of the serum would seem to depend on the introduction of suitable complement in conjunction with the amboceptors, or on the existence of such complement in the body of the patient.

Anticomplements.

Added proof of the multiplicity of complements has been obtained by experiments with anticomplements. As stated, the latter are obtained by immunization of suitable animals with normal or immune serums which contain complement or complementoid. When they are mixed with the homologous complements the haptophores of the latter are bound by means of the haptophores of the anticomplements. The evidence of this union lies in the fact that a complement which has been treated with its specific anticomplement is no longer able to activate the appropriate amboceptor. With properly selected serums, it may be shown that a given anticomplement will neutralize a complement which is specific for one amboceptor, but will have no effect on another complement which activates a different amboceptor. Hence, complements differ at least in this respect that not all have identical haptophores. Immunization with leucocytes, cells which contain complement, also causes the formation of anticomplement. Both natural and acquired antibacterial immunity may be lowered by the injection of anticomplement which is homologous to the complement of the animal.

Some time ago Ehrlich expressed the opinion that an amboceptor in certain cases may have more than one complementophilous haptophore; in other words, that it may be a polyceptor rather

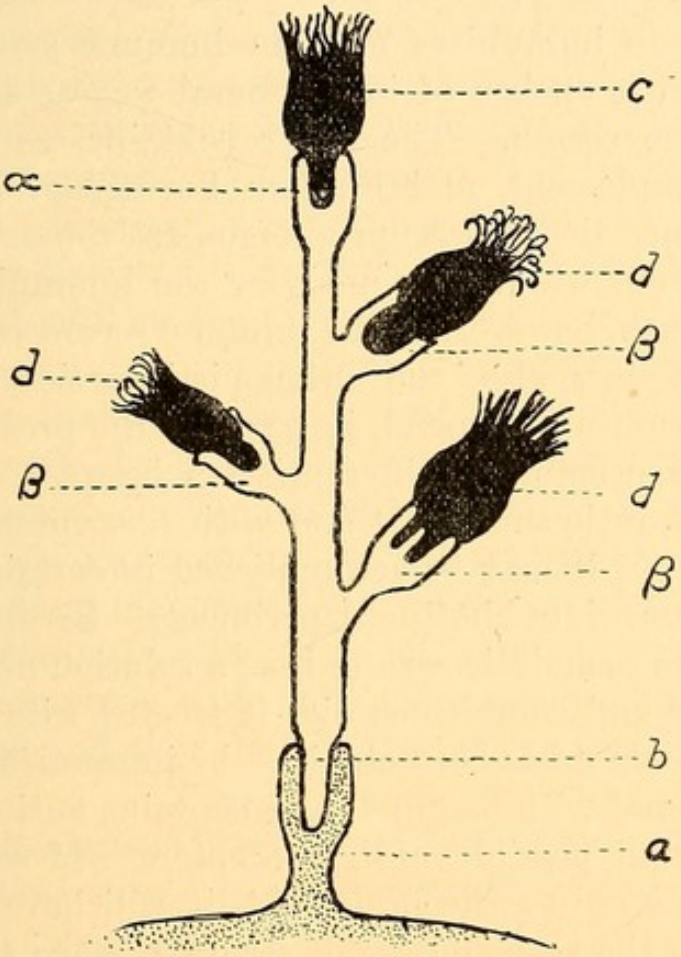


Fig. 8.—Illustrating the amboceptor with more than one complementophilous haptophore (a polyceptor). *a*, Cell receptor; *b*, cytophilous haptophore of the amboceptor; *c*, the dominant complement; *d*, the non-dominant complements α, The haptophore of the amboceptor for the dominant complement; β, those for the non-dominant complements. (From Ehrlich and Marshall.)

than an amboceptor. This has again been emphasized recently by way of explaining the ability of an amboceptor to absorb from a normal serum not only the complement which serves to activate the

amboceptor, but also others which happen to be present in the serum. The former is spoken of as the dominant complement and the latter as non-dominant complements. Figure 8 is an illustration of such a polyceptor.

Antiamboceptors.

If one immunizes with an immune serum the product is spoken of in a general way as an anti-immune serum. The latter contains, as stated, anticomplement, and through the agency of this substance the antiserum antagonizes the action of the serum which was used for the immunization. Inasmuch, however, as the immune serum contains amboceptors also, the antagonistic action of the antiserum may depend, in part, on the presence of antiamboceptors. Differentiation between the action of anticomplement and antiamboceptor is difficult, but it may be accomplished in certain cases by appropriate binding experiments. Serum 1, an inactive hemolytic serum, i. e., a solution of amboceptors and complementoid, is treated with serum 2. Serum 2 has been obtained by immunization of an animal with serum 1, and contains anticomplement and possibly antiamboceptors. If serum 2 contains only anticomplement, it will have no effect on the amboceptors of serum 1, when the two are mixed. The amboceptors are free to sensitize corpuscles which may be added, and the latter when sensitized undergo hemolysis in the presence of complement. If, however, serum 2 contains antiamboceptors, either the cytophilous or the complementophilous haptophore of the amboceptor will be bound. In either case corpuscles which are added subsequently would not appear as sensitized, for if the cytophilous haptophore had been bound by antiamboceptor union between cell receptor and

amboceptor could not occur; and if the complementophilous haptophore had been preoccupied complement would have no effect even if the amboceptors had united with the cells by their unbound cytophilous haptophores. Ehrlich and Morgenroth demonstrated such antiamboceptors for certain hemolytic serums, and it was their belief that they combine with the cytophilous rather than with the complementophilous haptophore of the amboceptor. However, Ehrlich has recently been able to prove the occurrence of an antibody for the complementophilous haptophore in one case. Pfeiffer also reports the demonstration of antiamboceptors for the specific amboceptors of anticholera serum. The possibility of antiamboceptor formation is one of practical bearing, in view of the fact that the prolonged treatment of a patient with a bactericidal serum may result in the development of such antibodies. If present in sufficient amount they would combine with new amboceptors which were injected and thus deviate the latter from the bacteria.

Danger of Formation of Antiamboceptors.

A phenomenon equally of theoretical and practical importance has to do with the so-called diversion (*Ablenkung*) of complement. It has been found that the action of a bactericidal or hemolytic serum is lessened, if a great excess of amboceptors over complement is added. To explain this fact Neisser and Wechsberg have supposed that when so many amboceptors are present that all can not be taken up by the cells, those which remain free are able to combine with some of the complement which is present and thus prevent the accession of the latter to the sensitized cells; that is to say, the complement is diverted from its natural course of

Diversion of Complement and Its Theoretical Danger.

action (Fig. 9). This amounts to a protection of the sensitized cells from the action of the complement. The phenomenon led Wechsberg to suggest that in the therapeutic administration of bactericidal serums it may be possible to give too much of the serum. Although diversion of complement is a demonstrated fact, its importance in serum therapy is perhaps not definitely settled.

Hemolytic Amboceptors of Venom.

It is of interest that amboceptors are widely distributed in the animal kingdom, and that in certain instances they may be demonstrated in the secretions. It has long been known that the

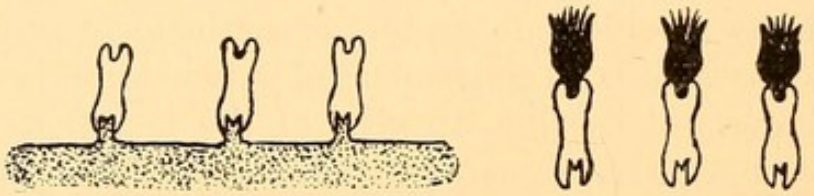


Fig. 9.—Illustrating diversion of complement. The free amboceptors have combined with the available complement, and thereby prevented the latter from activating the amboceptors which have united with the bacterial cell. (From Neisser and Wechsberg.)

venoms of serpents owe a great deal of their toxicity to their power of destroying red blood cells.* A given venom may contain several toxic substances, and the poisons of different serpents by no means coincide in their toxic properties. Cobra venom has at least two distinct toxins, one for the nervous tissue and one which dissolves erythrocytes, the neurotoxin having the greater pathogenic significance; it, moreover, agglutinates red blood corpuscles. The venom of the rattlesnake, on the other hand, is neurotoxic to a less degree, but has a pronounced influence in causing capil-

* See also part II, Chapter 3, concerning venoms.

lary hemorrhages. The latter power Flexner ascribes to a toxin for endothelial cells, which he calls hemorrhagin. Through the works both of Flexner and Noguchi and of Kyes, facts were learned concerning the hemolytic toxin of cobra venom, which may be of great importance in problems of general immunity. It seems that the hemolysin of venom is an amboceptor rather than a toxin of the usual nature, and that the aid of complement is necessary for its toxic action. The venom itself contains only the amboceptors, hence the toxicity of the substance depends on its being complemented after it is introduced into the body. The possession of suitable complement, therefore, is a source of danger in this instance rather than a means of protection for the individual. One may very well suspect that a similar relationship is possible in connection with other substances which are as yet unknown.

Endocomplement.

A fact of additional importance is that the amboceptor finds suitable complement not only in the serum of the animal but it may also be activated by a complement which the erythrocytes themselves contain. Kyes speaks of the latter as endocomplement, i. e., endocellular complement.

Lecithin.

In attempting to discover the nature of the complement which is present in the erythrocytes, various substances existing normally in the red cells, as cholestrin and lecithin, were obtained in pure form and their activating power for the cobra amboceptors was tested in reagent-glass experiments. From this work it was learned that lecithin, a definitely known chemical substance, has the activating power, and it was, therefore, assumed that the endocomplement of erythrocytes is nothing more

or less than lecithin. All erythrocytes contain lecithin, yet not all are equally susceptible to the action of venom in the absence of serum complement; that is to say, endocellular lecithin does not act as complement with equal readiness in all cases. In order to explain this variation it was necessary to assume that the lecithin in the cells of one animal may be more available as complement because it is bound to other cell constituents only in a very loose way, whereas in more resistant cells the union is of a firmer nature.

Cobra-lecithid.

The relationship between cobra amboceptors and lecithin seems to be a very definite one, for Kyes was able to obtain a union of the two without the intervention of erythrocytes. The resulting substance, the cobra-lecithid of Kyes, is a completed toxin and needs no further activation. We have yet to learn of the true nature of this new compound, the discovery of which seemed to augur a more intimate chemical knowledge of the substances which are concerned in immunity.

Hemolysis by the Combined Action of Colloids.

Lecithin is a colloid, and in this connection it is interesting to note that it may be used in combination with still another colloid in such manner that the hemolysis which they cause is analogous to that produced by hemolytic amboceptors and complements. Landsteiner tried the effect of colloidal silicic acid on erythrocytes which were entirely freed from serum, with the result that the corpuscles were agglutinated under its influence. It developed further, however, that colloidal silicic acid not only acts as an agglutinin, but also simulates a hemolytic amboceptor, and in the latter capacity it may be activated either by the ordinary complement of serum or by lecithin. Hence, we

have here an instance of the entire cytolytic action being performed by two known chemicals, which in their action appear to be analogous to amboceptors and complements. Yet even the action of these substances is obscure, for although the chemical formulæ of silicic acid and lecithin are sufficiently well known, the explanation of their activity as colloids is equally obscure with that of the albuminous substances.

Neutralization of Complement by Salts.

Another recent discovery which tends to bring the immune substances into closer touch with pure chemistry is that of Hektoen concerning the ability of certain salts (calcium chlorid, barium chlorid, etc.), to combine with complement in such a way that the latter loses its activating and combining function in relation to amboceptors. This was mentioned incidentally under the subject of antitoxins. The activity of the complement is again restored if the inhibiting salts are precipitated by suitable chemicals. The salts are used in such dilutions that they are largely ionized, and Manwaring believes their inhibiting action is due to the formation of compounds of the positive ions with the complement, resulting in such substances as Ca-complement, Ba-complement, etc. When the precipitating chemicals are added the ions are freed from this combination, as a result of which the complement recovers its activating properties. It has not as yet been determined whether variations in the salts in the fluids of the body cause changes in resistance by their action on native complements.

CHAPTER XIII.

CYTOTOXINS.

Following the discovery of immune hemolytic serums it was a short step to experiments which involved immunization with various other tissue cells, and as a result of such work we are to-day familiar with antiserums for almost every organ of the body.

Cytotoxin or Cytolysin.

Metchnikoff gave the name of cytotoxins to those serums which destroy cells other than bacteria and erythrocytes; the word cytolysin is used synonymously. Naturally a serum which destroys any cell whatsoever is cytotoxic, but according to the rather loose custom which prevails, we speak of bacteriolysins, hemolysins and other cytolysins, including among the latter serums which destroy leucocytes, the cells of the liver, kidney and other organs.

Theoretical Utility of Cytotoxins.

Cytotoxins are of interest, not only because they are produced in accordance with the general laws of anti-body formation, but they have, in addition, a certain theoretical and perhaps practical importance. Immediately on their discovery the possibility became manifest that they might be utilized in the elucidation of certain physiologic and pathologic problems. For example, by putting the thyroid out of function through injections of thyrotoxic serum it might be possible to confirm, or to prove incorrect, certain theories as to the rôle of the gland in metabolism. Or, by the selective destruction of a tissue, facts concerning its regenerative powers

might be learned. The use of an antipancreatic serum might throw some light on the nature of diabetes. Therapeutic possibilities also suggested themselves. One might be able by means of artificial anticytotoxic serums to counteract cytotoxins which were being formed pathologically in the body. Or, by injecting small amounts of a cytotoxin, perhaps one could stimulate to a renewed production of the homologous cells; small doses of a hemolytic serum might be useful in combating anemias. Or small amounts of leucotoxic serum might cause an increase in the number of leucocytes, and thereby an increased resistance to infection. Perhaps autocytotoxins are formed in some such manner as the following: An extraneous toxic substance causes the destruction of a few kidney cells, the constituents of the latter reach the circulation and stimulate other organs to the formation of autonephrotoxic amboceptors, which then assist in the destruction of more renal tissue, with the result that a vicious cycle is set up.

Lack of Specificity.

In spite of so many theoretical values, the study of cytotoxic serums has not yielded the results which were anticipated, perhaps chiefly because of their lack of specificity (Pearce and others). Although the cells of the different organs differ widely in their morphology and function, there are no doubt certain chemical constituents (receptors) which they possess in common. Of this we have experimental proof from the fact that immunization with one type of cell yields a serum which is toxic for the cells of various organs. It is difficult or impossible to injure one organ to the exclusion of all others by means of a cytotoxin. One may attempt to purify a cytotoxic serum through ab-

sorption of the adventitious amboceptors by means of the corresponding cells. Inasmuch, however, as the result is a decrease in the chief amboceptors as well as of the adventitious, the desired object is not fully realized. Theoretically the cytotoxic treatment of malignant tumors offers an important field for research. But here, too, various difficulties are involved, as lack of specificity of serums and the multiplicity of cell-types which constitute different tumors.

Determination of Cytotoxic Action.

Experiments with cytotoxic serums may be conducted *in vitro* or in the living animal. In either case a necessary condition for the recognition of the cytotoxic action is the presence of some distinctive sign of vitality on the part of the cell, the loss of which may be taken as evidence of cell-death. Loss of motility and of proliferative power indicate the death of bacteria, and solution of hemoglobin the death of erythrocytes. Under particular conditions loss of motility on the part of certain tissue cells, as spermatozoa, leucocytes and ciliated epithelium, is an evidence of cell death or cell injury. The toxic action of serums on cells of fixed form is more difficult to determine, and for evidence one must rely on such points as clearing of the protoplasm (digestion?), swelling of the cell and nucleus, actual solution of the cytoplasm, or degenerations of the homologous organs when the serum is injected into the living animal.

Technic of Immunization.

The technic of immunization with tissue cells is similar to that of immunization with bacteria. In order to obtain leucocytes in abundance, artificial leucocytosis is produced in the peritoneal or pleural cavity by the injection of bouillon, or lymph

glands, spleen or bone-marrow may be ground up and injected.

Immunization with solid organs, as liver, kidney or testicle, is easily accomplished, a necessary preliminary for injection being a thorough disintegration of the tissue by grinding with sterile sand; the resulting mass when suspended in salt solution passes through the injecting needle readily.

Amboceptors, Complements and Anticytotoxins.

Cytotoxins, like bacteriolysins and hemolysins, are complex substances, in that they consist of amboceptors and complements. The amboceptors alone are increased during immunization, the complement being a normal constituent of the serum of the animal. The phenomena of inactivation and reactivation are observable here as in connection with other cytolytic serums. Anticytotoxins are readily produced by immunization with many cytotoxins; the antiserum usually consists of anticomplement, but in some instances antiamboceptors have been described.

Spermotoxin.

Simultaneously, or nearly so, Landsteiner in Vienna and Metchnikoff in Paris reported the production of spermotoxic serums by immunization with spermatozoa, the natural motility of which rendered the recognition of cell death easy. The technic which Landsteiner first employed was that of the Pfeiffer experiment in that he immunized guinea-pigs with the spermatozoa of cattle and observed loss of motility on the part of the cells when they were injected into the peritoneal cavity of the immunized animals. Comparable with many other cytotoxins, spermotoxin kills the homologous cell without causing its solution. The loss of motility is also observed in hanging-drop preparations provided a fresh or a complemented

serum is used. Most normal serums show a greater or less degree of toxic action for the spermatozoa of other animals, and normal spermotoxins like the immune consist of amboceptor and complement. Metchnikoff claims to have produced an autospermotoxin by immunizing guinea-pigs with the spermatozoa of other guinea-pigs.

When a spermotoxic serum is injected into the living animal it is thought that the amboceptors are taken up by the homologous cells, and this would seem to affect the vitality of the spermatozoa, inasmuch as De Leslie rendered male mice sterile for 16 to 20 days by the injection of the serum.

It is of theoretical interest that castrated animals will yield spermotoxin by immunization, showing that the amboceptors are not of necessity produced by the analogous tissue of the immunized animal. From the fact that spermotoxic serums are hemolytic, it is assumed that certain receptors are common to erythrocytes and spermatozoa. Hemolytic serums, on the other hand, may not be spermotoxic. There is nothing contradictory in this lack of reciprocal action, for those receptors which are common to the two cells may not be important for the life of the spermatozoon, whereas the opposite condition prevails with the erythrocyte.

It is certainly of interest that immunization with the plasma of ova causes the formation of spermotoxic amboceptors, a fact which points to certain common constituents of the two cells.

Antispermotoxin may be produced by immunization with spermotoxic serum (anticomplement or antiamboceptor).

Following technic similar to that employed by Landsteiner, von Dungern obtained a cytotoxic serum for ciliated epithelium of the trachea. The cells disintegrated in the peritoneal cavity of the immunized animal, but not in that of the normal animal. This serum also proved to be hemolytic in spite of the fact that no erythrocytes were included in the injections. That the receptors which characterize ciliated epithelium are widely distributed is shown by the fact that immunization with cow's milk causes the formation of a cytolytic serum for the tracheal epithelium of the cow.

Cytotoxin for Ciliated Epithelium.

Leucotoxic, lymphotoxic or lymphatotoxic serums are prepared by immunization with exudates which are rich in leucocytes, or with the emulsions of lymphoid organs: lymph glands, spleen, bone marrow. Metchnikoff prepared the first serum of this nature by the injection of the spleen of rats into guinea-pigs. Leucotoxic serums are toxic, not only for leucocytes, but also for red corpuscles and endothelial cells. When injected into the peritoneal cavity the endothelium is thrown off, and when given subcutaneously the capillary endothelium is attacked, with the result that blood escapes to form a large hematoma. The action of the serum on leucocytes may be observed *in vitro*. The mononuclear cells are often more susceptible than the polymorphonuclears, although this depends somewhat on the animals and the particular organ used for immunization. The cells lose their motility, the cytoplasm becomes transparent, and swells to form a large clear vesicle, which appears to be surrounded by a sharp, thin membrane. The cell contents may be discharged or entirely liquefied, the nucleus alone being rec-

Leucotoxin.

ognizable. Leucocytes are agglutinated by the serum. A strong leucotoxic serum may be fatal to the animal when injected into the peritoneal cavity or blood stream, the exact cause of death being obscure.

Old Age. Metchnikoff, taking the view that the phenomena of old age depend on the destruction of various tissue cells by the mononuclear leucocytes (macrophages), expressed the hope that a lymphotoxic serum might be utilized to combat the action of these cells with the result that life would be prolonged. Whether or not his view as to the cause of old age is correct, his plan of antagonizing it had to be abandoned because leucotoxic serums do not injure the macrophages to the exclusion of other leucocytes.

Effect of Leucotoxic Serum on Resistance to Infections. The injection of a leucotoxic serum into the peritoneal cavity of a guinea-pig causes a temporary decrease in the number of leucocytes, and during this period of hypoleucocytosis the resistance of the animal to peritoneal infections with the organisms of typhoid and cholera is lowered. One may refer this effect to the destructive action of the serum on the leucocytes, by which phagocytosis is prevented, or, according to Wassermann, it may depend on the action of anticomplement which the leucotoxic serum contains. (Leucocytes contain complement, hence immunization with leucocytes causes the formation of anticomplement.) It is probable that both factors are of influence. In the course of 24 to 48 hours after peritoneal injection of the serum, the leucocytes reaccumulate to an enormous extent. During this secondary hyperleucocytosis resistance to peritoneal cholera or typhoid is increased. Some non-toxic substances,

as bouillon, have a similar effect, and although the secondary leucocytosis is never so great as that caused by the leucotoxic serum, the protective action is equally high. It would seem that, leucocyte for leucocyte, those which accumulate following the injection of leucocytoxic serum are less efficient in antibacterial action than those whose presence is caused by nontoxic substances. (Ricketts.) Hence there probably is no field for leucotoxic serum as a means of temporarily increasing resistance to bacterial infections.

By guarded immunization Besredka obtained an antileucotoxic serum.

Nephrotoxic Serum.

Nephrotoxic serums have been brought into close relationship with clinical and anatomic problems by a number of investigators. Some normal serums are held to be nephrotoxic inasmuch as their injection is followed by albuminuria and renal degenerations. Immune nephrotoxins have a similar but more pronounced effect, and Lindeman referred the death of his experiment animals to the development of a uremic condition. Of more than ordinary interest is the claim of certain workers that autonephrotoxins may be formed in the body. One (Lindeman) caused a toxic nephritis in dogs by the injection of potassium-bichromate. The serum of this dog, although free from chromic acid, was toxic for other dogs, producing the symptoms which are caused by an immune nephrotoxic serum. It was supposed that the chromic acid in the first dog caused disintegration of renal cells and that the constituents of the latter were then taken up by nephrotoxic receptors which normally reside in the organs of the animal; as a result the receptors were overproduced

Autonephrotoxins.

and their presence became manifest when the serum was injected into other dogs. In accordance with this view the original toxic cause of a degenerative nephritis would be of less consequence for the continuance of the condition than the formation of the nephrotoxic amboceptors; i. e., the formation of an autonephrotoxin.

Somewhat similar results were obtained by others through ligation of the renal vein or artery on one side. Constituents of cells of the isolated kidney were supposed to be absorbed, and as a consequence nephrotoxic amboceptors were produced in excess by organs of uncertain identity. To the action of the new-formed bodies were attributed the degenerative changes which were found in the opposite kidney, and the nephrotoxic properties which the serum manifeested when injected into a healthy animal of the same species.

Antinephrotoxin, Cardiac Hypertrophy.

According to Ascoli and Figari unilateral nephrectomy so injures the opposite kidney (overwork) that the serum of the animal becomes nephrotoxic. They state also that an animal, the serum of which contains nephrotoxin, may antagonize the latter by the production of antinephrotoxin, and suggest that spontaneous recovery from nephritis may be due to the action of such an antibody. They would account for the cardiac hypertrophy of nephritis by the action of nephrotoxic serum in causing contraction of the peripheral vessels with consequent increase of blood pressure; and for the nervous symptoms on the basis that the serum contains a neurotoxic constituent.

We hardly dare consider such far-reaching conclusions as decisive until they have been extensively confirmed. Yet whatever may be their real value

they serve to emphasize the possibility that those principles which are so important in relation to immunity against infectious diseases, may be equally important in relation to other pathologic conditions.

Hepatotoxins.

Hepatotoxins have been obtained by a number of workers, and the attempt has been made to produce autohepatotoxins by injecting liver tissue of the guinea-pig into animals of the same species. The success was not unqualified. Hepatotoxins when injected are reported to cause insular degenerations of the liver; however, the lesions may be caused, in part at least, by capillary emboli of endothelial cells or erythrocytes.

Neurotoxin.

Neurotoxic serums have been studied with some thoroughness. Whether one injects the cerebrum, cerebellum or spinal cord, the resulting serums apparently are similar; either an anticerebral or an anticerebellar serum will cause degenerations of the spinal ganglion cells. In view of their broad range of action it seems improbable that neurotoxic serums will be of service in clearing up the etiology of system degenerations of the nervous tracts. They are usually hemolytic and hemagglutinating and may also be endotheliotoxic and leucotoxic. When mixed with emulsions of the homologous brain tissue the neurotoxic amboceptors are bound by the receptors of the nervous tissue, and the serum consequently loses its toxicity. Antineurotoxic serums have been described.

Cyncytiotoxin in Relation to Eclampsia.

Syncytiolysin is the name given to a serum which is obtained by immunization with the placenta. Certain writers (Veit and Scholten, Charrin and Delamare) report that the injection of placentar tissue alone causes albuminuria, a consideration

which led them to assume that the placentar cells contain a nephrotoxic substance. Inasmuch as placentar cells or their constituents may reach the circulation during eclampsia (Schmorl) it was not a long step to suppose that the nephritis of pregnancy is due to the toxic syncytial cells which are absorbed. The results which Weichardt reported gave some strength to the view just cited. By treating placentar tissue of rabbits with the specific cyncytiolysin the toxin supposedly was liberated, and the mass when injected into normal rabbits is said to have produced symptoms of an eclamptic nature. On the basis of these observations the hope has been expressed that an antitoxin for eclampsia might be prepared by immunization with placentar tissue. However, the conditions are by no means simple; any value which the destruction of circulating syncytial cells or their toxin would afford might be more than offset by the action of the hemotoxin and neurotoxin which the serum is said to contain. Whether or not the hypothetical toxin of syncytial cells may be separated from the other cell constituents, and whether immunization with the toxin will yield an antitoxic serum are possibilities which remain for further investigation; the results cited have not been obtained by all observers.

Liepmann hopes for a serum-diagnosis of pregnancy. If, as supposed, the blood of a pregnant woman contains syncitial cells or their products of degeneration, the serum when mixed with a specific syncytiolysin may cause a precipitate. He claims to have demonstrated the presence of placentar constituents in the circulation by this biologic method.

Thyrotoxin.

Antithyroid serum is prepared by immunization with ground-up thyroid tissue or with extracts of the organ. It is hemolytic, even though all the blood has been washed from the tissue which was injected. Portis immunized with the "colloid" material of the gland obtaining a hemolytic thyrotoxic serum. When injected into the living animal degenerative changes are produced in the thyroid, and some authors report the tetanic phenomena which often follow surgical removal of the thyroid. In very careful work, however, Portis could not produce "the exact picture presented by thyrodectomized animals." Degenerative changes were found in various organs, as liver, spleen and kidneys.

Sympathetic Ophthalmia.

Brown Pusey has made the interesting suggestion in regard to sympathetic ophthalmia that the disease may be due to the formation of autocytotoxins which are specific for the cells of the inner surface of the ciliary body and iris. The disintegration products of the corresponding cells in the eye which was primarily injured would constitute the stimulus to the formation of the specific antibodies. The possibility is as yet a problematic one.

Pancreotoxin.

The experimental study of cytotoxic serums for the pancreas has, up to the present time, thrown little light on pancreatic diseases. It stated that the serum may cause transient glycosuria, and it is said to have an antitryptic action in experiments performed in the test-glass.

Other Cytotoxins.

The results of different observers concerning the action of antiserums for the adrenal gland are not in entire accord. Although degenerative changes may be caused in the gland when the serum is in-

jected, the action is not specific; the serum may be highly hemolytic (Abbott).

Ceni claims to have demonstrated in the circulation of epileptics a cytotoxin which causes the epileptic attacks, and reports the production of a specific antitoxin.

Toxin of Exhaustion.

Weichardt has published descriptions of a toxin which is peculiar to states of exhaustion, giving an account of the specific antitoxin which he produced by immunization.

Other cytotoxins which have been prepared, as those for the pituitary body, gastric mucosa and cardiac muscle, have at the present time nothing more than general biological interest.

Concerning Autocytotoxins.

It would seem that no question in relation to cytotoxic serums is more important than the possibility that autocytotoxins may develop and institute the vicious cycle which was mentioned earlier. It is true that the results of some investigators suggest the probability of such a process, but it would be going too far to say that its existence as an important pathologic law has been established. On the contrary, the development of autocytotoxins is one of the rarest of occurrences in experimental work; and Ehrlich has spoken of the inability of the body to form such antibodies as a condition of "horror autotoxicus." The cells of our kidneys and our erythrocytes certainly do degenerate, and it is quite possible that the receptors which are thereby liberated actually reach cytotoxic amboceptors which are situated in other organs. In the event that the process extends to this point, Ehrlich assumes that the amboceptors are of a sessile nature, that in spite of the stimulation to which the cells are subjected the "sessile

"Horror Autotoxicus."

amboceptors" may not be overproduced and liberated as in the case of antibodies for bacterial substances or for the cells of other species. In accordance with this explanation we are saved from intoxications of the nature in question because of the sessile nature of the cytotoxic amboceptors.

CHAPTER XIV.

PHAGOCYTOSIS.

As one may learn from the writings of Metchnikoff, phagocytosis, in its broad sense, exercises three distinct functions: nutritional, resorptive and protective.

Phagocytosis for Purposes of Nutrition.

Phagocytosis, for purposes of nutrition, is most highly developed in unicellular ameboid organisms, but is found also in animals of considerable organic differentiation. It is, perhaps, nowhere more striking than among certain myxomycetes, which are large, naked, multinucleated, protoplasmic masses belonging to the plant kingdom, and which possess a peculiar, slow, undulating motility. Ingestion is accomplished through protoplasmic arms (pseudopodia) which are thrown out to envelop the object. Minute plant and animal cells, living or dead, are ingested in this manner by the myxomycetes, amebæ and other unicellular organisms and are subsequently digested by means of intracellular ferments. The ferments which have been extracted from such cells are proteolytic since they digest gelatin and fibrin, usually in an acid but sometimes in an alkaline medium; that from amebæ has been called amibodiastase. In the process of digestion a "vacuole," acid in reaction and containing the ferment, forms around the ingested particle. In certain phagocytic unicellular organisms the protoplasm shows a degree of differentiation, a mouth and an anus being simulated at points where the food is most readily taken in and discharged. Instances are cited in which ameboid

organisms protect themselves against inimical cells by ingesting, killing, and finally discharging or digesting the latter.

Chemotaxis.

The botanist, Pfeiffer, first described the phenomena of negative and positive chemotaxis in relation to the myxomycetes. Under certain conditions they either are attracted toward or move from moist places. That a negative chemotaxis may be changed into a positive was shown in relation to salt solutions. When placed in the vicinity of or in contact with strong solutions the cell recedes, whereas if one passes gradually from weaker to stronger solutions the latter eventually attract rather than repel the cell.

Intestinal Phagocytes.

As one goes higher in the animal scale intracellular digestion for purposes of nutrition is confined to rather definite groups of cells. The intestinal epithelium of certain invertebrates consists of "sessile phagocytes," cells which, individually or after fusion into plasmodial masses, surround and digest solid particles of food. It is said that in sponges the digestive tract is not sharply separated from the mesodermal tissue, and the cells of the latter share with the former the function of intracellular digestion.

In higher invertebrates and in all vertebrates the intestinal epithelium ceases to be essentially phagocytic, digestion being accomplished rather by ferments which have been secreted by the intestinal and related glandular epithelium. Such animals, nevertheless, possess an abundance of phagocytic cells, but they are in the main mesoblastic in nature, and may have nothing more than a remote relationship to the nutrition of the organism.

Macrophages and Microphages.

Metchnikoff divides the phagocytic cells of vertebrates into the macrophages and the microphages. The macrophages or large phagocytes include the large lymphocytes, endothelial cells, ameboid connective tissue cells and others which may occasionally take up foreign particles. Our polymorphonuclear leucocytes are the microphages. In relation to immunity we are concerned chiefly with the large lymphocytes (macrophages), and the polymorphonuclear leucocytes (microphages). Although such cells may contain many ferments, Metchnikoff recognizes but one type in relation to their resorptive, digestive and bactericidal activities. This he calls cytase and distinguishes that of the macrophage as macrocytase and that of the microphage as microcytase. Cytase corresponds to the complement of Ehrlich. The two cells do not have identical activities, the macrophage being concerned specially in the resorption of tissue cells and in immunity to certain chronic diseases, as tuberculosis and leprosy, whereas the microphage is the cell which is conspicuously antimicrobic in relation to acute infections.

Cytases.

Resorption of Native Cells.

According to Metchnikoff, the leucocytes are very active in the resorption of useless or foreign cells. During the metamorphosis of certain invertebrates it is said that the larval tissues are englobed and digested by wandering phagocytic cells. In involution of the uterus the muscular tissue is invaded by leucocytes which take up and digest or carry away the "retrogressive elements." Metchnikoff's conception of certain atrophic processes, particularly

those which are grouped among the senile atrophies, is of interest to pathologists. In sclerotic atrophy of the ovaries the large lymphocytes invade the tissue, surround and destroy the ova and follicular epithelium and eventually, as fibroblasts, participate in the formation of fibrous tissue which to a degree is substituted for the original structure. In old individuals or in those of failing mentality it is said that ganglionic cells are found in a greater or less degree of atrophy because of the action of certain mononuclear phagocytes (neuronophages) which are contiguous to or form a zone around the cell. The neuronophages may represent mononuclear cells from the blood or those of proliferated neurogliar tissue. The best examples of this condition were found in very old dogs. The chromophores of the skin, according to Metchnikoff, may be considered as chromophages. Whether or not they are of epithelial origin, as he claims, they are said to exist normally in the hairs in a latent or inactive condition. As old age comes on, or as a result of other obscure causes, their attitude becomes an active one, and they proceed to take up and digest the normal pigments of the hairs. Hence, white hairs are the result of an autoparasitism by certain mononuclear phagocytes. In muscular atrophy it is held that the sarcoplasm takes up the striated tissue after the manner of phagocytes.

The Whitening of Hairs.

We come into closer touch with our general subject of immunity when we consider the resorptive function of the phagocytes for cells which are foreign to the host, for example, toward erythrocytes which are injected for the purpose of producing a

Resorption of Foreign Cells.

hemolytic serum. Following such an injection into the peritoneal cavity there occurs a great accession of macrophages which ingest the erythrocytes, dissolve the hemoglobin and eventually digest the stroma. The same phagocytes are involved in the resorption of any other foreign cells of animal origin which may be injected. In view of the intracellular hemolysis by the leucocytes, one may suspect that the latter contain a hemolytic ferment; one which, perhaps, is analogous to the hemolysin (hemolytic amboceptors and complement) of serums. On this point there has been sharp discussion. Metchnikoff cites observations to show that a ferment of this nature may be extracted from the lymphoid organs, that it contains a heat-susceptible constituent, and that when fresh it may be used to reactivate a heated hemolytic serum. This would indicate that the leucocytes contain cytase (complement), but it is not clear that they would also contain the fixators (amboceptors). Nevertheless, the demonstration of an intraleucocytic hemolysin and a knowledge of the phagocytic power of the leucocytes for erythrocytes form the basis for Metchnikoff's belief that serum-hemolysin is nothing more than intraleucocytic hemolysin, which under proper conditions may reach the serum or plasma. By an extension of this conception it is held that all cytolysins are produced by the macrophages.

Formation of Cytotoxins.

Thermostabile Hemolysin from Organ Extracts.

Korschun and Morgenroth, on the other hand, obtained from lymphoid and various other organs, not a thermolabile hemolysin, but one which withstands prolonged boiling—a coctostabile hemolysin which is soluble in alcohol, shows no amboceptor-

complement composition, and is incapable of yielding antihemolysin by immunization. These results, Metchnikoff holds, are only in apparent discord with those obtained by himself and his pupils, and depend on the methods of extraction which were employed. In order to obtain the thermolabile hemolysin uncontaminated with the thermostabile, the extraction must be a rapid one. If, on the other hand, it is prolonged, as Metchnikoff assumes that of Korschun and Morgenroth to have been, the intracellular ferments digest the remaining cell constituents, including the thermolabile hemolysin, and the thermostabile hemolysin is liberated or formed in the process.

Believing that cytase, under normal conditions, exists only within the leucocytes, and that its presence outside these cells is artificial, Metchnikoff cites experiments similar to the following in support of his views:

Cytase an Intracellular Substance.

Given a guinea-pig which has been immunized with the blood of a goose: if fresh goose corpuscles are injected into the peritoneal cavity, the cells are hemolyzed in the fluid without the occurrence of phagocytosis. Two explanations of the extraleucocytic presence of cytase and fixators, which is indicated by this result, are possible: first, that they are present normally and continuously in the plasma of the immunized animal, or, second, that they become liberated at the time the corpuscles are injected. According to Metchnikoff, the latter contention prevails rather than the former. He recognizes a phenomena which bears the name of phagolysis, i. e., solution, partial or complete, or phagocytes. Almost any foreign substance or fluid which one

Phagolysis.

Liberation of Cytase by Phagolysis.

may choose to put in contact with leucocytes so stimulates or injures them that they discharge certain of their constituents. If the fixators and cytase are among the constituents which are discharged at the time the injection is made, the extracellular hemolysis encountered in the experiment described above might depend on the liberation of these substances rather than on their natural occurrence in the plasma. If this be true, and if one could in some way fortify the leucocytes against phagolysis, the plasma would remain free from hemolytic power. Metchnikoff accomplishes such fortification, i. e., prevents phagolysis, by a simple procedure, which demands nothing more than the peritoneal injection of a small quantity of bouillon or salt solution twenty-four hours in advance of the experiment. Possibly by this means the leucocytes have been habituated to the presence of a foreign fluid, or the new leucocytes which accumulate possess greater resistance. Whatever the explanation, the erythrocytes which are injected at this critical time are said not to undergo extracellular hemolysis, but instead are englobed and dissolved by the macrophages. These results and others of a similar nature are the basis for the belief that cytase normally is intracellular, and that it becomes extracellular only when the leucocytes are subjected to injurious influences. The fact that the serum of defibrinated or coagulated blood contains cytase is not in discord with such an opinion, for in this instance also the leucocytes may be injured to such a degree that certain of their constituents are discharged. We are well aware that fibrin-ferment is liberated under these circumstances.

Prevention of Phagolysis.

Fixators Produced by Leucocytes.

It was equally desirable, if possible, to determine the relation of fixators to the leucocytes. The situation is, however, very complex, and, although Metchnikoff regards the fixators as secretion or excretion products of phagocytic cells, the question is, perhaps, not definitely settled. When phagolysis is prevented in the manner described, the injected erythrocytes may well absorb fixators from the plasma and still undergo no hemolysis until englobed by the phagocytes. It is considered that fixators in contrast to cytase may exist in the circulating plasma.

Phagocytosis in Immunity.

Phagocytosis as a feature of local resistance against microbic invasion was considered in relation to inflammation. We come now to speak of the relationship of the leucocytes to general states of immunity, having reference to the conditions which have been designated as natural and acquired antibacterial immunity, and natural and acquired antitoxic immunity.

The first expressions of Metchnikoff concerning the antimicrobic activity of phagocytes, the power of freeing the organism from "invaders of every sort," were made altogether from an *a priori* standpoint in an address delivered in 1883, "Ueber die Heilkräfte des Organismus." He justified his position on general grounds, having in mind the "more general phenomena of phagocytosis and the resorption of corpuscular elements," as he had observed them in various zoölogical studies.

Natural Immunity to Bacteria.

Shortly there came to him the opportunity of studying an infectious disease among the Daphnia (water-flea), a small transparent crustacean. The disease was caused by a blastomyces which forms

a long needle-shaped spore. After being swallowed by the animal the spores penetrate the intestinal wall into the body cavity where they are surrounded, englobed and digested by the white blood corpuscles. If this occurred with sufficient vigor all the spores were disposed of and the animal recovered. Sometimes, however, the spores germinated even after they had become intracellular, and when the parasitic cells reached maturity they apparently had the power of killing the leucocytes through the agency of a secretion peculiar to themselves. In the event that the latter process was sufficiently extensive the tissues were soon overrun with parasites and death resulted from a septicemic condition. The observations were made in the living transparent animal.

Natural Immunity.

Although the example cited seemed convincing, it was, of course, necessary that observations should extend over many infectious processes before phagocytosis as the cause of natural immunity could be accepted as a general fact. This has been done on rather broad lines by Metchnikoff and his pupils, and the results have served to convince them that the phagocytes are responsible for natural immunity in all instances, and that the degree of natural immunity in a given case depends on the degree of phagocytosis which is manifested against the organism. As stated previously, the microphage, with its microcytase, is held responsible for antibacterial immunity in most instances, although the macrophage is concerned in certain chronic infections.

If an animal is susceptible to a virulent culture of anthrax, but resistant to a weak culture, the

Relation of Phagocytosis to Virulence of Bacteria.

phagocytic power is found to be greater for the weaker organism. The highly virulent culture creates a condition of negative chemotaxis, with the consequence that leucocytes are not attracted and microbic proliferation proceeds rapidly. Without going into details, studies of the following and perhaps other micro-organisms have strengthened Metchnikoff in his views: staphylococci, streptococcus, pneumococcus, gonococcus, vibrio of cholera in infections of the guinea-pig, the vibrio of goose septicemia in relation to the guinea-pig, which is naturally immune, the spirillum of relapsing fever, tubercle bacillus, yeast cells and other fungi, and certain animal parasites (*Trypanosoma lewisii*).

Toxins as Cause of Negative Chemotaxis.

Most important are certain conditions which create a condition of negative chemotaxis, or otherwise engage the phagocytes so that they refuse to take up the essential organism. Vaillard says that all animals are immune to pure cultures of the tetanus bacillus or its spores, provided the latter have been washed entirely free of toxin. The absence of toxin permits of positive chemotaxis and phagocytosis, whereas toxin when present causes negative chemotaxis, and the bacilli proceed to further toxin formation. The same is held to be true in infections by some other organisms.

Accidental Engagement of Phagocytes.

It seems definitely established that contaminating organisms (pyogenic cocci, *Bacillus prodigiosus*) may greatly increase the virulence of the bacillus of symptomatic anthrax, *Bacillus aerogenes capsulatus* and the tetanus bacillus—anaërobic organisms. On the one hand, the secondary bacteria may produce more favorable conditions for

the growth of the anaërobes by consuming local oxygen, or, as Metchnikoff believes, they may so engage the phagocytes that the latter have no disposition to take up the essential organism. This condition may be an important one in other mixed infections, as when the streptococcus complicates diphtheria and scarlet fever.

Acquired Immunity to Bacteria.

If the phagocytic power is an index of the degree of natural antibacterial immunity, is the same correspondence to be recognized when the immunity is acquired? To answer this question satisfactorily it is necessary to bring phagocytosis in relation to two different types of antibacterial immunity which it is possible to recognize. Cholera is an example of that type of antibacterial immunity in which the bactericidal power of the serum undergoes a great increase. It is stated that anthrax represents another type in which the immunity is not dependent on the bactericidal power of the serum. Probably the same may be said of acquired immunity to the streptococcus, staphylococcus and the pneumococcus, yet it is perhaps not definitely established that the immunity in these instances is antibacterial rather than antitoxic. For the present we may, however, with Metchnikoff, consider that the immunity is antibacterial and that it is a cellular or phagocytic immunity.

Anthrax.

Rabbits which have been immunized against anthrax respond to subcutaneous or intraperitoneal injection of a virulent culture by concentrating so vast a number of microphages at the site of inoculation that the fluid becomes purulent in appearance. Examination shows an enormous degree of phagocytosis. When, on the other hand, non-im-

mune rabbits are submitted to similar inoculations, the fluid which accumulates locally is of a clear serous character, contains few leucocytes, and no phagocytosis is observable; the animals die of a rapidly developing septicemia. From the results one may well suspect that the immunity is related to and perhaps coextensive with the acquired phagocytic power.

Phagocytes Take Up Virulent Bacteria.

But is the serum of no influence? It has often been held that phagocytes take up bacteria only after the latter have been injured or killed by the serum or plasma. Metchnikoff answers this objection experimentally by inoculating an immune rabbit with anthrax, withdrawing some of the exudate at a time when phagocytosis is complete, and injecting it into a non-immune rabbit. The second animal dies. Since none but phagocytized bacilli were injected into the non-immune rabbit (!), and since the latter succumbs to anthrax, it seems not only unnecessary, but unjustifiable, to assume that the bacteria must be attenuated by the serum before they can be taken up by the leucocytes. May the serum, nevertheless, have some obscure action which may not be included under such terms as bactericidal and attenuating? It seems fairly well established that anti-anthrax serum, at least from certain animals, may exert a protective influence when injected into other animals in conjunction with or in advance of the culture; yet Metchnikoff discredits the importance of such protection and says that "those properties of the body fluids, as the bactericidal, preventive and agglutinating, fall away into the background in such examples of immunity." It is the tendency

The Influence of Serum.

of the school of Metchnikoff to refer the protective power of a serum to its faculty of stimulating the phagocytes rather than to its effect on the micro-organisms. We shall see, however, in speaking of opsonins (p. 193) that even in relation to anthrax the serum may possess a distinct property which facilitates phagocytosis, not by stimulating the phagocytes but by some action on the bacteria.

Cholera and Similar Infections.

Concerning those diseases in which immunity is characterized by a great increase of the bactericidal amboceptors or fixators, Metchnikoff does not disregard the existence or importance of the immune bodies, but rather seeks to show that they are a product of phagocytic activity. The conditions are held to be similar to those already mentioned in connection with *intra vitam* hemolysis. That is to say, microcytase exists only in the leucocytes of the immune animal under normal conditions; it escapes into the plasma, or into the serum during coagulation, only as a consequence of the phagolysis already mentioned. The phenomenon of Pfeiffer occurs only because the injected culture injures the leucocytes, resulting in the liberation of microcytase, which in conjunction with the fixators causes the solution of the vibrio. When phagolysis is prevented by a preceding injection of bouillon, phagocytosis and intracellular solution of the organisms entirely supplant extracellular solution.

Intravascular Phagocytosis and Phagolysis.

If an immune animal receives an intravascular injection of the vibrio of cholera and is sacrificed shortly, the relation of the organisms to the leucocytes may be studied in stained microscopic sections of the organs (lungs). Leucocytes which

have undergone phagolysis are seen to be clumped in the pulmonary vessels and in their immediate vicinity one finds many micro-organisms which have been changed into the characteristic granules by the action of the cytase which has escaped from adjacent leucocytes. Coincident with the phenomenon of phagolysis, the leucocytes lose their phagocytic power; hence, no bacteria are found within the leucocytes. On the other hand, all those vibrios which are remote from the leucocytes have a perfectly normal appearance. Phagolysis in the blood stream may be prevented, just as in the peritoneal cavity, by a preceding injection of bouillon into the vessels. In this instance when the culture is injected no extracellular solution or transformation of the organisms into granules takes place, but as in the peritoneal cavity, their destruction is accomplished entirely within the microphages. Metchnikoff holds to the correctness of these observations and interpretations, although contradictory results were obtained by Pfeiffer and his pupils. As further evidence that cytase does not exist normally in the plasma Metchnikoff cites the condition which is found in the anterior chamber of the eye in immune animals. The vibrios continue unaffected in the aqueous humor until such a time as leucocytes wander in, when they are destroyed by phagocytosis. Hence, cytase does not exist in the aqueous humor, and if not in the aqueous humor it is surely absent from the plasma; for if present in the plasma it would reach the anterior chamber by a process of diffusion. Similar conditions prevail in edematous fluids. In another instance a portion of a vein,

Microcytase is Intracellular.

filled with blood, was resected and centrifugated without the formation of a clot (absence of phagolysis); the plasma contained no cytase. Also Gengou collected and centrifugated blood in tubes which were coated with paraffin, and thus avoided clotting; here also cytase was absent from the plasma.

Increase of Fixators and of Phagocytic Power.

It would seem, then, that two important antibacterial factors characterize immunity to cholera and similar infections: the development of specific fixators, and a greatly increased phagocytic power on the part of the leucocytes. Metchnikoff leans to the view that bacteria, having absorbed fixators, are more readily phagocytized, but no clear idea is given as to the change which the fixators produce. However, he would not refer the increased phagocytic power entirely to the influence of the fixators. He believes that the leucocytes of the immune animal have *per se* a higher phagocytic power than that of the normal animal. In anthrax, for example, the phagocytic power is heightened in spite of the fact that there is no increase in specific fixators. This view, however, is opposed by Denys and Leclef, who found that the leucocytes of the immune animal, when transferred to normal serum, had no greater phagocytic power than normal leucocytes.

Fixators Product of Microphage.

Metchnikoff believes that fixators, like cytase, are produced by the microphage. That the lymphoid organs may form certain fixators seems probable from the observations of Pfeiffer and Marx in regard to cholera and Wassermann and Takaki in typhoid. During the process of immunization and at a time when amboceptors were absent from

the serum they could be demonstrated in the blood-forming organs (spleen, lymph glands, bone-marrow). Metchnikoff suggests that they may be produced in these organs by the microphages which have wandered in after having englobed the micro-organisms. In contrast to cytase the fixators readily abandon the leucocytes which produced them and become a constituent of the plasma.

Natural Immunity to Toxins.

The leucocytes have also been brought in relationship to antitoxic immunity and the formation of antitoxins. In experimental tetanus exudates which are rich in leucocytes contain more toxin than does a similar quantity of blood. That is to say, the leucocytes have the power of absorbing toxins, and it is held that the natural immunity of the animal depends on the degree to which this power is present. The immunity of the chicken to tetanus depends not on non-susceptible nerve cells nor on the presence of natural antitoxin, but on the absorbing power of the leucocytes for the toxin. Not only do leucocytes absorb toxins, but it is held that they also are the producers of antitoxins. As compared with the side-chain theory, it is a peculiarity of the view of Metchnikoff that antitoxin does not represent a constituent of the tissue cells, but rather the toxin itself, which has been altered by leucocytic activity in a manner as yet obscure.

Passive Antitoxic Immunity.

In passive antitoxic immunity the idea of a chemical union between toxin and antitoxin does not meet with general acceptance among the upholders of the phagocytic theory. It is sometimes said that antitoxins are efficacious from the fact that they stimulate phagocytosis (absorption) of

the toxin, the latter then suffering disintegration in the leucocytes.

Summary. The following statements summarize the phagocytic theory of immunity as conceived by Metchnikoff:

1. Natural immunity to bacteria depends on and is coextensive with phagocytosis and subsequent digestion of the microbes. Intraleucocytic destruction of the micro-organisms is accomplished by the cytase, possibly aided by intraleucocytic fixators. Normal serum is devoid of both fixators and cytase.

2. Acquired immunity to bacteria depends on the establishment of a heightened phagocytic power as the result of immunization or infection. In diseases like anthrax, in which fixators are not increased, this new power is an acquired property of the leucocytes and is independent of any influence on the part of the serum. In diseases like cholera, the new fixators which are formed may render the micro-organisms more susceptible to phagocytosis, but this is probably secondary to increased function on the part of the phagocytes. Both cytase and fixator are produced by the phagocytic cells. In acquired active immunity to bacteria the fixators may be free in the serum and plasma, but the cytase is intracellular. In all cases cytase becomes extracellular only as the result of phagolysis.

3. In passive immunity to bacteria, as when an antibacterial serum is injected for the sake of prophylaxis or cure, the serum is efficacious chiefly because it stimulates the leucocytes to increased phagocytosis.

4. Natural immunity to toxins depends on the power of the leucocytes, and perhaps the generative organs, to absorb the toxin.

5. Active immunity to toxins is established through the activity of the leucocytes, by which the toxin is probably so changed as to constitute antitoxin.

6. In passive antitoxic immunity the antitoxin presumably acts by stimulating the phagocytes to an increased absorption of the toxin.

Opsonins.

Many investigators are carrying on work in regard to phagocytosis and the properties of serums from a point of view which is entirely unbiased. From sources of this nature discoveries of recent date indicate that phagocytosis of micro-organisms by the leucocytes is impossible without the aid of some property in the serum. It seems that these substances, which the discoverers, Wright and Douglas, call opsonins, act directly on the bacteria, and that there is no reason to suppose that their virtue lies in a stimulation of the phagocytes themselves. The facts which permit of this deduction are the following: 1. When the fresh defibrinated blood of some animal is mixed with the culture of a suitable micro-organism (staphylococcus, streptococcus, anthrax bacillus, etc.) and placed in the thermostat for 20 or 30 minutes, stained preparations of the mixture show that the polymorphonuclear leucocytes contain a large number of the microbes. 2. If, however, all the serum is washed from the blood before adding the micro-organisms, practically no bacteria are ingested. This shows the importance of the serum, but does not differentiate between some effect on the leucocytes, on

the one hand, or the bacteria, on the other. 3. In order to decide this point one may subject the suspension of bacteria to the action of fresh cell-free serum, and after a contact of about 30 minutes remove all the serum by centrifugation, and mix the "sensitized" culture with serum-free blood; phagocytosis occurs almost to the same degree as when the fresh defibrinated blood, containing serum, is used. These results seem to show definitely that phagocytosis depends on the power of the opsonins to affect the bacteria in some peculiar manner. Opsonins are very susceptible to heat, and, like complement, disappear spontaneously from the serum in a short time. Hektoen and Ruediger, and Bulloch and Atkin have confirmed the observations of Wright and Douglas and the former have added facts of importance. It seems that opsonin has a structure like that of toxin, i. e., a haptophorous and an opsoniferous group; "by heating sensitized bacteria the opsoniferous group appears to be destroyed, but the inactive opsonin (opsonoid) by saturating the receptors of the bacteria prevents further sensitization by fresh serum" (Hektoen and Ruediger). Various salt solutions neutralize the opsonins. Opsonins will be brought into still more important relationship to phagocytosis if it can be shown definitely that they are increased as the result of immunization or infection.

CHAPTER XV.

THE SIDE-CHAIN THEORY OF EHRLICH AND ITS RELATION TO THE THEORY OF PHAGOCYTOSIS.

Side-Chain Theory Applied to Nutrition.

In 1885, before the discovery of toxins and antitoxins and before there was any knowledge as to the real nature of immunity, Ehrlich[1] published a small volume on the "Oxygen Requirements of the Body." Herein the belief was expressed that the assimilation of foods by cells is accomplished only after chemical union has taken place between the food substance and some constituent of cellular protoplasm. It is not the understanding that assimilation is at an end, however, when this union has occurred, for certain molecules of complex chemical nature and of great size must be split up into simpler substances before they can enter into the composition of protoplasm. Therefore, the cell constituent which combines with the nutritious molecule serves only as a link to bring the foodstuff into relation with the digestive, oxidizing or fermentative activities of the cell.

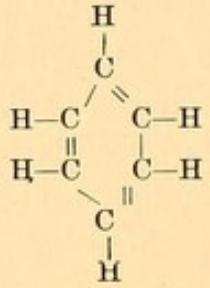
"Leistungskern" and Side-Chains.

Ehrlich speaks of that portion of living protoplasm which represents the cellular activities as the *"Leistungskern"* of the cell, the center of cellular activity, or the central group of the protoplasm, whereas those chemical groups which bind the food substances are called the side-chains of the *"Leistungskern."*

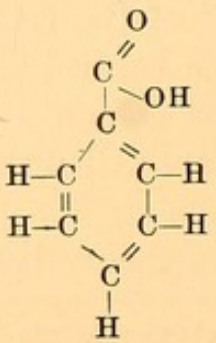
The author of the theory has made his concep-

1. Ueber das Sauerstoffbedürfnis des Organismus.

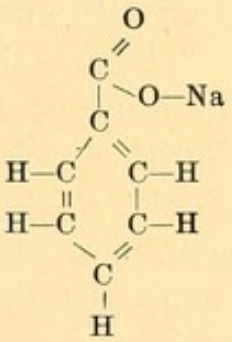
tion more tangible through an analogy which was drawn with the so-called ring or nucleus of benzol and its side-chains. The molecule of benzol, C_6H_6, has a definite formation in which each carbon atom is linked to two others in such a manner as to form a ring; three valences of each carbon atom are satisfied in this way, and the fourth is satisfied by atoms of hydrogen, one of which is attached to each carbon atom, thus:



This ring is analogous to the "*Leistungskern*" of the cell. A great variety of chemical compounds exists and very many may be produced synthetically by substituting for one or more atoms of hydrogen, one or more other groups of atoms which may be very simple or very complex. The groups which have been substituted are called side-chains. Thus benzoic acid is formed from benzol by substituting the acid radical COOH for a particular H, and the COOH in this instance is a side-chain of the ring:



Just as the side-chains of the "*Leistungskern*" may combine with food particles, so may the side-chains of the benzol ring combine with other groups of atoms and thereby assimilate the latter, so to say, into the ring. To choose a simple example, the sodium of sodium hydroxid may unite with the side-chain COOH to form sodium benzoate, the hydrogen of the acid radical being replaced by the sodium, thus:



Presumably it is in some such manner as sodium is brought into relationship with the benzol nucleus, in the example cited, that the food substances are brought into relationship with the "*Leistungskern*" of the cell.

Haptophores.

The hypothesis of Ehrlich carries with it the assumption that the side-chains of a cell possess or consist of definite groups of atoms capable of uniting chemically with certain other definite groups of atoms in the food particles; hence both the side-chain and the food substance have combining groups—haptophores. The side-chains of the cells Ehrlich now calls receptors, elements which we have already recognized in connection with immunity. Inasmuch as different foods have different chemical compositions, it is likely that their binding groups are not identical; and if this be

true there must exist many kinds of receptors each of which is able to unite only with that food substance which has a corresponding binding group of atoms.

In contrast to the condition with respect to foods, it is held that chemical substances of known composition, drugs and alkaloids never become incorporated as a part of the protoplasm, that is, they do not unite with cell receptors, although they may affect the vitality and function of protoplasm profoundly. Their inability to yield antibodies as a result of immunization is supposed to depend on this condition. Such substances, according to Ehrlich, exist in the cell in a condition of unstable salt formation with some constituent of the protoplasm, or in a state of solid solution.

The following statement from a recent publication by Ehrlich summarizes the nutritional aspect of the theory: "We must assume that all substances which enter into the structure of protoplasm are fixed chemically by the protoplasm. We have always distinguished between assimilable substances which serve for nutrition and which enter into permanent union with the protoplasm, and those which are foreign to the body. No one believes that quinin and similar substances are assimilated, that is, enter into the composition of the protoplasm. On the other hand, the food substances are bound in the cells, and this union must be considered as chemical. One can not extract a sugar residuum from cells with water, but must first split it off with acids in order to set it free. But now such a chemical union, like every synthesis, demands the presence of two binding

groups of maximal chemical affinity, which are suited one to the other. The binding groups which reside in the cells and which bind food substances I designate as side-chains or receptors, while I have called those of the molecules of foodstuffs the haptophorous groups. I also assume that protoplasm is endowed with a large series of such side-chains, which through their chemical constitution are able to bind the different foodstuffs and thereby provide the prerequisite for cellular metabolism."

Side-Chain Theory Applied to Immunity.

If the side-chain theory of nutrition is to become the side-chain theory of immunity it is necessary that it undergo elaboration in order that the formation of antibodies may be adequately explained. If, as Ehrlich assumes, the union of toxin with cell receptors causes the overproduction of the latter as antitoxin, and if this union is analagous to that of food substances with similar receptors, one may wonder that antibodies are not formed for our ordinary foods, antibodies which would be discharged from the cells and which would unite with circulating nutritious particles and thereby bring about a condition of starvation. Without entering into the intricacies of this question, it seems probable that normally a condition of physiologic equilibrium exists between the food substances on the one hand and the cellular activities on the other, so that the union of food with protoplasm constitutes no abnormal stimulus to the *"Leistungskern"* of the cell. When, however, cells are diverted from their normal metabolic function by union with toxins and other "abnormal food substances," the effect on the cell is de-

scribed as a cell defect, the defect consisting of the functional elimination of the receptor. The *"Leistungskern"* as the vital or regulating center of the cell repairs the defect by the formation of new receptors, and in harmony with the hypothesis of Weigert produces not only enough to repair the defect, but a great excess, with the result that many are thrown into the circulation. The analogy of the *"Leistungskern"* with the benzol ring can not be carried to this extent, for the latter has no power of reproducing side-chains to take the place of one which has been bound by some new group of atoms.

Essential Tenets of Ehrlich's Theory.

It will be appropriate in this place to consider the character of the proof which has been offered in support of the three tenets which constitute the framework of the theory of Ehrlich. These three tenets may be expressed as follows: 1. Antitoxins counteract toxins by entering into chemical union with them; a similar union takes place between other antibodies and their homologous substances. 2. Toxins in injuring cells combine chemically with a definite constituent of the protoplasm, the cell receptor; other antigenous substances[2] enter into similar union with the appropriate receptors of cells. 3. The specific antibodies of the serum are new-formed receptors identical in structure with those which, as cell constituents, had combined with the homologous antigens.

Chemical Union of Antibodies with Antigens.

First tenet: In the early days of studies on immunity (1890-1897), the action of a toxin and the efficacy of an antitoxin could be determined only

2. An antigen or an antigenous substance is one which is able to cause the formation of an antibody.

by injecting these substances into living animals, and the animal experiment naturally continues to be the means of testing the curative and prophylactic values of serums. So long, however, as such experiments were performed exclusively in the living animal the nature of the action of antitoxin remained to a certain extent in doubt. It remained uncertain whether antitoxin is protective because it actually destroys the toxin, because neutralization of a chemical nature occurs, or because in some manner it increases the resistance of the inoculated animal. In Chapter viii, p. 78, experiments were cited to show that antitoxin does not destroy the toxin, and this is generally admitted today. There continues to be some difference of opinion, however, in relation to the two other possibilities, i. e., as to whether antitoxin combines chemically with toxin, or is efficacious because of its stimulating power on the tissues of the animal. Behring, the discoverer of antitoxin, was from the beginning an exponent of the chemical theory, even at a time when the conceptions of Ehrlich had not been fully developed. On the other hand, certain noted investigators, especially Roux and Buchner, and later Metchnikoff, stood for the alternative view.

Ricin and Antiricin.

Following closely on Behring's great discovery, Ehrlich studied the hemagglutinating toxin ricin, from the castor-oil bean, and by immunization with it produced a specific antitoxin, i. e., antiricin. Ricin is toxic to erythrocytes both in the animal body and in the test-tube, and if it could be shown that antiricin protects in the test-tube by a direct effect on the toxin, it was highly prob-

able that its action in the animal body would be of a similar nature. The results left no doubt in the mind of Ehrlich that antiricin unites chemically with ricin, and the applicability of this principle in animal experiments became all the more apparent when it was shown that the proportion of antiricin which protects *in vitro* also protects *in vivo*. It is held that similar proof of chemical union between bacterial hemolysins, the hemolysin of venom and the leucocidin of the staphylococcus with their respective antitoxins is equally valid.

Chemical Nature of the Neutralization of Toxins by Antitoxins.

Although the animal body can not be dispensed with in testing the action of the antitoxins of diphtheria and tetanus, certain principles of chemical action are found to prevail which leave no doubt in regard to the chemical neutralization of the toxins. If neutralizing proportions of diphtheria toxin and antitoxin be mixed in a test-tube and injected immediately, the serum does not afford absolute protection; if, however, the mixture is allowed to stand for from fifteen to twenty minutes before injection, the protection is absolute. This alone would point to an action of the antitoxin on the toxin, for the completion of which a certain amount of time is required. For the complete neutralization of tetanus toxin by its antitoxin about forty minutes are necessary at ordinary temperatures. Then certain other chemical principles described in Chapter viii, p. 79, are found to hold true: That neutralization proceeds more rapidly at higher than at lower temperatures, more rapidly in concentrated than in dilute solutions, and that it takes place in accordance with the law of multiple proportions.

Granting, then, that neutralization of toxin by antitoxin is of a chemical nature, the first essential step in the chemical or side-chain theory is established. If antitoxin combines chemically with toxin, union must occur through combining groups which each molecule possesses. Herein lies the experimental justification for assuming the existence of haptophorous groups.

Union of Agglutinin and Amboceptors with Cell Receptors.

The situation is more difficult in regard to the union of receptors of the second and third orders, i. e., agglutinins and amboceptors with the homologous receptors of bacteria and other cells. One can not titrate bacteria against agglutinin or bactericidal amboceptors so exactly as toxin can be titrated against antitoxin, for, in the first place, it is difficult to obtain at will a desired concentration of bacteria and to keep it without alteration, and, in the second place, bacterial cells contain many more receptors than are necessary for their agglutination and solution. A given mass of bacteria will take up varying quantities of agglutinin, depending on the concentration of the latter, and the same principle applies to the absorption of bactericidal and hemolytic amboceptors. As more and more agglutinin is added, the total amount absorbed increases with each addition, although the ratio of absorbed to unabsorbed agglutinin grows less continuously. The conditions which govern this phenomenon are not understood. Perhaps no condition speaks more decisively for chemical union of these bodies with cell receptors than immunization experiments which were carried on with cells which had been treated with a great excess of the specific antiserum. The as-

sumption was made that if one could force all the receptors of erythrocytes, for example, to take up the specific amboceptors, such corpuscles should lose their power to cause the formation of a hemolytic serum when injected into a suitable animal. This would follow logically, for the receptors of the corpuscles, being already bound, would not be free to unite with receptors of the immunized animal. Antibodies were not formed under these circumstances, from which it is concluded that the receptors of the erythrocytes had united chemically with the antibodies of the serum (Sachs). In order to completely occupy all the receptors of the vibrio of cholera Pfeiffer used 3,000,000 to 4,000,000 times the dissolving amount of the anti-cholera serum. Although the mere absorption of agglutinins and amboceptors by the homologous cells is cited in favor of the chemical hypothesis, we may bear in mind the contention of certain investigators that this absorption is physical rather than chemical.

Chemical Nature of Union of Toxins and Other Antigens with Cell Receptors.

Second tenet: What evidence have we that toxins and other antigenous substances enter into chemical union with receptors in the cells of the immunized animal? It is probable that no observation speaks more strongly in favor of such union than a famous experiment of Wassermann's in which the central nervous system of guinea-pigs was ground up with tetanus toxin, the mixture allowed to stand for a short time and then injected into mice. The mixture was found to be non-toxic, and further experiments showed that the neutralizing power resides in the solid tissue in the emulsion. It is claimed by Ehrlich that

this experiment demonstrates positively that chemical union of tetanus toxin takes place with constituents of the nervous tissue. The toxin having been completely neutralized can not again be extracted from the tissue. The condition is the opposite in relation to some poisonous alkaloids, as strychnin, which it appears does not combine with the protoplasm firmly and may again be extracted by simple methods.

Von Dungern conducted very important work with the precipitins, which is interpreted as showing that albuminous substances other than toxins are taken up chemically by the cells. He injected considerable quantities of a foreign serum into the veins of rabbits and studied its disappearance from the blood of the injected animal. Traces of the foreign serum could be recognized by treating the rabbit serum with a specific precipitin for the former, the precipitin having been obtained previously by the immunization of other animals. The foreign serum disappeared from the circulation of the rabbit with some rapidity and since it could not be demonstrated in the excretions, it seemed necessary to assume that it had been bound by the cells, that is to say, by the cell receptors.

Proliferation of Receptors.

Third tenet: Is there any direct experimental proof that those constituents of cells which have been designated as cell receptors actually undergo multiplication in the cell itself as a preliminary to their discharge into the circulation in the form of antibodies? If this condition could be demonstrated in one instance, one might reasonably consider that it typifies a law according to which all antibodies are formed. Further experiments by

von Dungern with the precipitins seem to show that such intracellular overproduction actually does occur. The experiments concern the fate of "Majaplasma" (plasma of the crawfish) when injected into the circulation of the rabbit (see above). If a single injection of the serum is given, a specific precipitin for the latter body in due time may be demonstrated in the serum of the rabbit. Eventually the precipitin disappears from the circulation by excretion or other means. At that time, when all the precipitin has disappeared, one may assume that the cells of the animal still contain an increased number of precipitin receptors, although the latter are no longer produced to such an extent that they are thrown into the circulation. If this condition exists the tissues of the animal at this time should be able to absorb a larger amount of the foreign serum, given in a second injection, and perhaps absorb it more rapidly than the tissues of an untreated rabbit. Using a specific precipitating serum in order to detect traces of the foreign serum which still remained in the blood of the injected animal, von Dungern determined that its tissues actually do absorb the plasma more rapidly than do the tissues of the untreated rabbit. The cells of the former have a greater absorbing power, i. e., a greater binding power for the plasma; therefore, an increased number of receptors.

These examples are, perhaps, sufficient to illustrate the principles of experimentation which have been followed in the attempt to obtain definite proof of the correctness of the essential points of the theory. The results are in entire accord with

the primary assumptions and show that the theory continues to serve as an explanatory basis for newly-discovered facts, and as a foundation on which new researches may be instituted.

Other Important Principles of Ehrlich.

In addition to the three main principles treated of above, the following points are necessarily included in a summary of the views of Ehrlich, many facts of a corroborative nature having been ascertained in independent laboratories.

1. The recognition of different types of tissue receptors by which peculiarities in the action of the different antibodies are explained. Receptors of the first order, as antitoxins, anticomplements and antiamboceptors, are regarded as relatively simple bodies because no other constituent can be recognized than the haptophorous group by which they combine with their homologous substances. Receptors of the second order are more complicated in that they have something more than the mere binding power; usually they are able to produce some observable change in the substance with which they unite. Hence, each has a toxophorous or a zymotoxic group in addition to the haptophorous, and the two groups are part of the same molecule. Toxins, agglutinins, precipitins and complements are receptors of the second order. Receptors of the third order, i. e., the bacteriolytic, hemolytic and cytotoxic amboceptors, are still more complex in that they are, so to say, only partial antibodies, the complete body consisting of the amboceptor-complement complex. The amboceptor is not an active body, but serves as an intermediary body to connect the active substance, complement, with the cell. In the cytolytic proc-

ess the amboceptor through its cytophilous haptophore first unites with the cell, and as a result acquires an increased affinity for complement, with which it unites through its complementophilous haptophore. Only after this double union is completed may complement affect the cell. From this it follows that complement in the cytolytic process does not combine with the cell directly. As previously stated, Bordet and others oppose the idea that the absorption of these bodies is of a chemical nature, considering it rather to be a physical process.

Ehrlich has intimated his belief that tissue amboceptors play the chief rôle in the fixation of foods by the cells of the body.

2. The chemical theory explains the specificity which characterizes the formation and action of antibodies. Every antigen has a haptophore which is different from those of other antigens; consequently, it unites only with the corresponding cell receptor, and the latter when overproduced and cast into the circulation retains its specific binding power for the corresponding antigen.

3. The multitude of antibodies which have been obtained indicate that the cells contain a vast number of different receptors which correspond to the three types now recognized; that is, there is a different antitoxin receptor for every kind of toxin, etc.

4. Ehrlich has limited the application of the term toxin to those substances of animal or plant origin, immunization with which causes the formation of specific antitoxins. Other characteristics have been given in Chapter vii, p. 65.

5. Receptors of the second order, toxins, agglutinins, precipitins and complements, undergo a peculiar degenerative change, spontaneously or as a result of exposure to injurious agents, in which the toxophorous or zymotoxic group disappears or is rendered inactive. The termination -oid is affixed to the altered bodies, as toxoid, agglutinoid, precipitoid and complementoid. Wechsberg has described a similar degeneration of one of the haptophores of amboceptors, calling the product amboceptoid. Toxoids and complementoids on immunization cause the formation of corresponding antitoxins and anticomplements, by virtue of retention of their haptophorous groups.

6. By means of a special technic devised for studying the neutralization of toxin by antitoxin, i. e., the partial saturation method, Ehrlich found diphtheria toxin to be a very complex substance. Not all the molecules of the toxin have the same affinity for antitoxin, and according to the degrees of their affinity have received the names of prototoxin, deuterotoxin and tritotoxin. Similarly, protoxoids and syntoxoids are molecules of toxoid having different affinities for antitoxin. These conditions are represented graphically by means of the "toxin spectrum" described previously.

7. Ehrlich claims that the diphtheria bacillus secretes two toxins, one of which causes the acute manifestations of diphtheritic intoxication, whereas the second toxin, i. e., toxon, has a prolonged incubation period and probably causes diphtheritic paralysis. Toxon has a lower affinity for diphtheria antitoxin than the other constituents of the

toxin solution, but is neutralized by the same antitoxin. This view is strongly opposed by Arrhenius and Madsen, who, working on the basis that the neutralization of toxin takes place according to certain laws of physical chemistry, claim that toxon is nothing more than toxin which has dissociated from the toxin-antitoxin molecule.

8. It is thought that the incubation period which characterizes the action of toxins represents to a large degree the time required for the action of the toxophorous group after the toxin has been bound by the cells.

9. Ehrlich stands for the multiplicity of complements in opposition to Bordet and others who claim the existence of but one complement (alexin). The various complements differ in the nature of their haptophores, without regard to possible differences in their zymotoxic groups.

10. Only those organs which have suitable receptors may produce an antibody for a given antigen, i. e., only those cells which may enter into chemical combination with the antigen. It does not follow, however, that only those organs which show clinical or anatomic lesions may produce, say, an antitoxin; for other organs not so susceptible to the action of the toxin may still possess the suitable receptors and cast them out as antitoxin.

Causes of Different Types of Immunity.

The various types of immunity are explainable on the basis of the side-chain theory in the following terms:

1. Natural immunity to toxins may depend on (*a*) a lack of suitable cell receptors, the toxin consequently finding no point of attack; (*b*) a very

low affinity between cell receptors and toxin so that the latter does not unite with the cells except under special conditions (e. g., the immunity of chicken to tetanus); or (*c*) on the presence of natural antitoxins.

2. Acquired active antitoxic immunity depends on the multiplication and excretion of cell receptors (antitoxin) into the circulation, the new-formed bodies having the power of combining chemically with additional toxin which may be introduced.

3. Passive antitoxic immunity, as established by the injection of an antitoxin, depends on the ability of the antitoxin to combine chemically with the toxin and thus to divert the latter from the cells.

4. Natural immunity to bacteria depends on (*a*) a lack of suitable cell receptors with which the toxic bacterial constituents might combine; (*b*) a very low affinity between cell receptors and the toxic bacterial constituents; or (*c*) on the presence of natural bacteriolysins (amboceptors and complements).

5. Acquired active antibacterial immunity depends on the multiplication and excretion into the circulation of specific cell receptors (amboceptors) which have the power of uniting with complement to kill the micro-organisms which may be introduced.

6. Passive antibacterial immunity, as established by the injection of a bacteriolytic serum, depends on the ability of the amboceptors contained in the serum to unite chemically with the receptors of the micro-organism, as a result of which complement is absorbed to kill and perhaps

to dissolve the bacteria. The complement may be present in the serum which is injected, or the natural complement of the individual may be utilized by the amboceptors.

Comparison of Theories of Ehrlich and Metchnikoff.

When one seeks to compare the theory of Ehrlich with that of Metchnikoff, one finds little more in common than the general purpose of explaining the phenomena of immunity. Yet it is remarkable that where there is so little in common there are so few contradictions of an essential nature.

The theory of Ehrlich has that degree of definiteness which it must have in order to be a plausible chemical theory, whereas that of Metchnikoff seems more general in that it is so largely biologic and vitalistic.

Each has a certain relation to nutrition. Phagocytosis as a nutritional measure is found in lower types of animals, and accomplishes nothing further than to bring the food substance in contact with the digestive ferments contained in the cell. In relation to nutrition the theory of Ehrlich begins, so to say, where the phagocytic theory leaves off, involving, as it does, the method by which food substances become a part of the protoplasm.

Metchnikoff, with Ehrlich, recognizes the various antibodies which have been discovered. The former holds that all are produced by the phagocytes without suggesting clearly a method by which they may be formed. Ehrlich assumes a very precise method by which they may be formed, but designates no particular cells as their producers, stating only in a general way that an antibody is produced only by those cells with which the

antigen may combine; in some instances, the leucocytes may be such cells.

The theory of Metchnikoff is not concerned with the structure of toxins and the various antibodies, nor with the method by which toxins may injure the cells, whereas Ehrlich presents definite conceptions on these points.

Both recognize that there is more than one complement (cytase). Ehrlich recognizes no limit to the varieties which may exist, whereas Metchnikoff describes but two cytases, microcytase and macrocytase.

The view which Metchnikoff has expressed, that antitoxin is produced by some action of the phagocytes on the toxin, is directly opposed to that of Ehrlich which recognizes antitoxin as a product of the cell itself.

They agree that amboceptors (fixators) become extracellular in the blood.

Metchnikoff holds that complements (cytases) are produced only by the phagocytes and that these substances are found in the plasma or serum only as a result of injury to the phagocytes (phagolysis). These points are not involved essentially in the theory of Ehrlich. Certain investigators who work in harmony with the side-chain theory, as well as those who represent the views of Metchnikoff, have extracted complement from the leucocytes. Some of Ehrlich's supporters believe that complement exists normally in the plasma.

Metchnikoff and Ehrlich hold divergent views concerning the action of antitoxins, the former believing that antitoxins stimulate the phagocytes to an increased absorption and consequent destruc-

tion of the toxin, whereas Ehrlich claims that antitoxin neutralizes toxin by combining chemically with it.

According to Metchnikoff, all types of immunity depend, directly or indirectly, on phagocytic activity. While the side-chain theory is not in harmony with such a broad assumption, it carries with it no denial of the phenomenon of phagocytosis nor of its importance in certain infections.

Compatibility of Theories.

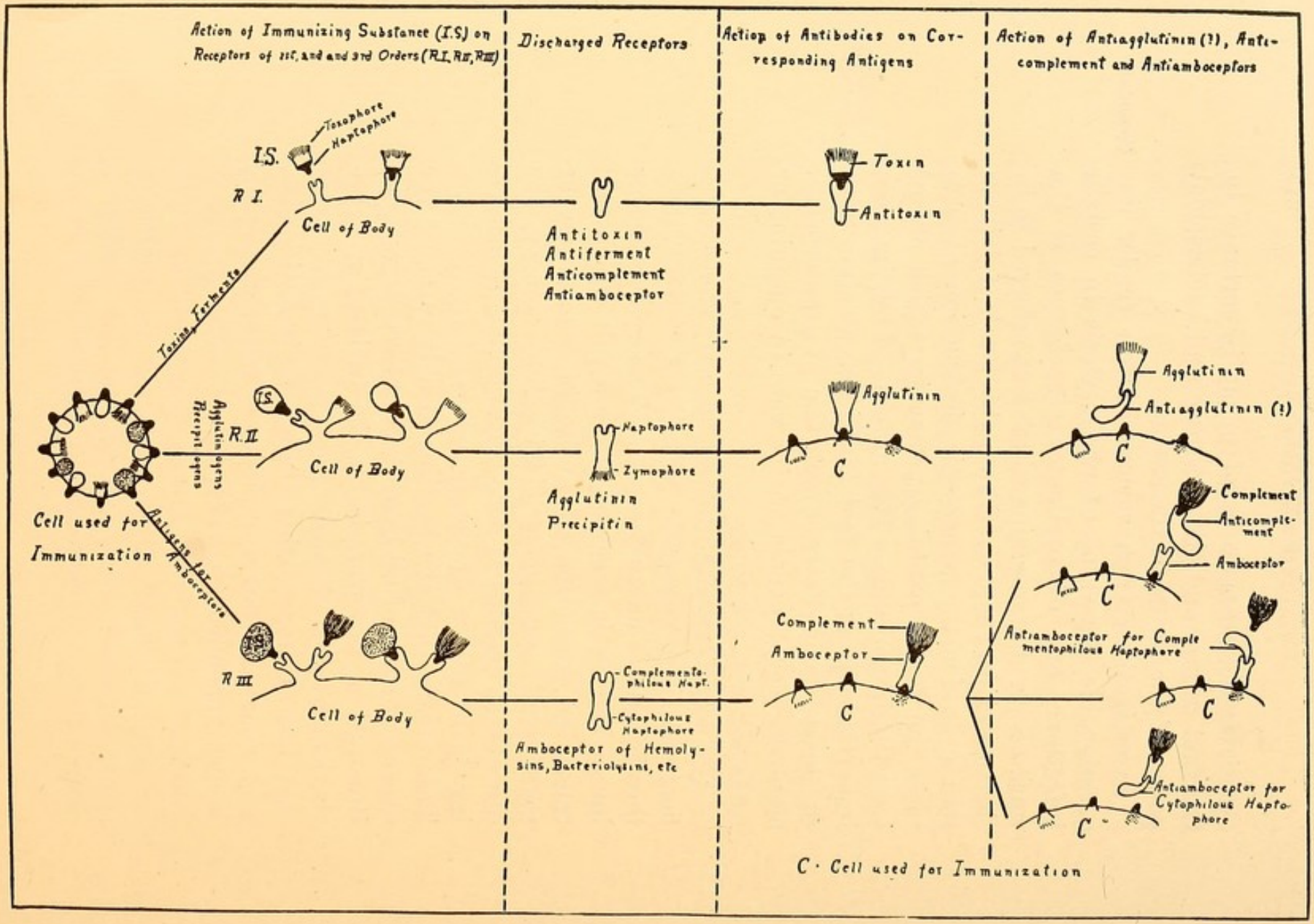
From these selected considerations it is seen that the two theories do not stand to each other in the relation of antitheses, and in the light of present knowledge it would seem unwarranted to cling to one view to the absolute exclusion of the other. It does not follow that because demonstrable serum properties explain immunity to one disease, or to a certain group of diseases, that recovery from all diseases must depend on properties of the serum; nor because phagocytic activity explains recovery in certain instances that recovery from all diseases must depend on a similar activity. The conditions which exist in each disease, of course, must be recognized independently. It so happens that recovery from a certain group of diseases, e. g., staphylococcus, streptococcus and pneumococcus infections, is not accompanied by the development of conspicuous antitoxic or bactericidal properties in the serum, but they are characterized by a great increase in the number of circulating leucocytes (microphages), cells of known phagocytic and bactericidal power, whereas the opposite conditions are found in certain other diseases, e. g., typhoid and diphtheria. If one seeks the most apparent explanation in each case, the great leuco-

cytosis would seem to be of prime importance in the first group, and the antitoxic and bactericidal power of the serum in the second.

Investigations from various sources render unquestionable the value of phagocytosis in certain infections, and of particular significance is the work concerning opsonins which was referred to in the preceding chapter. From this work it follows that even for the phagocytic destruction of bacteria the serum contains properties which are of essential importance. This appears of all the more importance from the fact that immunization with at least some micro-organisms (streptococcus, staphylococcus) causes an increase in opsonins or bacteriotropic substances. **Opsonins.**

The accompanying illustration, with some modifications, is taken from "Ehrlich's Seitenkettentheorie," by Ludvig Aschoff. The cell used for immunization is assumed to be a cell which will cause the formation of antitoxin, agglutinin or precipitin, and bactericidal amboceptors; the diphtheria bacillus is such an organism, considering toxin as one of the receptors of the bacillus. This means that the bacillus is able to cause the overproduction of all three types of receptors. The illustration, however, is on the basis of a hypothetical cell (p. 216).

A list of immunizing bodies, their anti-bodies, and synonyms for complement and amboceptor, is also appended (p. 217).


Action of Immunizing Substance (I.S.) on Receptors of 1st, and and 3rd Orders (R.I, R.II, R.III)
Discharged Receptors
Action of Antibodies on Corresponding Antigens
Action of Antiagglutinin (?), Anticomplement and Antiamboceptors
Toxophore
Haptophore
I.S.
R.I.
Cell of Body
Toxins, Ferments
Antitoxin
Antiferment
Anticomplement
Antiamboceptor
Toxin
Antitoxin
Agglutinogens
Precipitogens
R.II
Cell of Body
Haptophore
Zymophore
Agglutinin
Precipitin
Agglutinin
Antiagglutinin (?)
C
Cell used for Immunization
Antigens for Amboceptors
Complement
Anticomplement
Amboceptor
R.III
Cell of Body
Complementophilous Hapt.
Cytophilous Haptophore
Amboceptor of Hemolysins, Bacteriolysins, etc
Complement
Amboceptor
Antiamboceptor for Complementophilous Haptophore
Antiamboceptor for Cytophilous Haptophore
C · Cell used for Immunization

LIST OF IMMUNIZING BODIES AND THEIR ANTIBODIES.

List of Immune Substances.

Antigens or Immunizing substances.	Products of immunization.		
Toxins.	Antitoxins.		
Complements	Anticomplements		
Ferments.	Antiferments		
Precipitogenous substances	Precipitins		
Agglutinogenous substances	Agglutinins		
Opsinogenous substances of bacteria	Opsonins		
Cytotoxin producing substances	Cytotoxins....	Hemolysins Bacteriolysins Special Cytotoxins Spermotoxin Nephrotoxin Hepatotoxin Neurotoxin Syncytiolysin, etc.	Consisting of two bodies, i. e., complement and amboceptor.

IMMUNIZATION WITH ANTIBODIES.

Precipitins Agglutinins Cytotoxins Hemolysins, etc.	Antiprecipitins Antiagglutinins (?) Anticytotoxins Antihemolysins, etc.	Consisting either of anticomplements or antiamboceptors; the latter may be an antibody for the complementophilous or for the cytophilous haptophore of the amboceptor.

SYNONYMS.

Complement	Amboceptor
Alexin	Immunkörper
Cytase	Zwischenkörper
	Intermediary body
	Substance sensibilisatrice
	Fixator
	Preparator
	Copula
	Desmon

CHAPTER XVI.

PRINCIPLES OF SERUM THERAPY.

In the strict sense serum therapy means the injection of antitoxic or antibacterial serums for curative or prophylactic purposes; this is passive immunization or direct serum therapy. Active immunization, in which the tissues of the individual are induced to form antitoxins or antibacterial substances as a result of vaccination or protective inoculations, may be considered as indirect serum therapy. We may, therefore, include the latter as one of the serotherapeutic measures.

Bearing in mind the significance of the terms active and passive immunization, and the fact that they may be used for curative and prophylactic purposes, the various procedures may be classified as follows:*

I. PROPHYLACTIC INJECTIONS.

Classification of Serotherapeutic Measures.

A. Active immunization, in which vaccination and protective inoculations are included, as with the organisms of typhoid, cholera and plague. Depending on the material injected, the result is the formation of antitoxins or antimicrobic substances (amboceptors); agglutinins are formed incidentally.

1. Inoculation of virulent organisms. (*a*) Inoculation with small amounts of a virulent organism, i. e., of a non-fatal dose; used principally in experimental work. (*b*) Inoculation with virulent

* Modified from Deutsch and Feistmantel in "Die Impfstoffe und Heilsera," Leipsic. Geo. Thieme, 1903.

organisms into a tissue which has some natural resistance. The success of vaccination against smallpox by using virus obtained directly from the diseased, a method which was practiced in earlier times, was probably due to the fact that the virus found unfavorable conditions for the development of virulence in the skin. In some instances immunization is accomplished more successfully by inoculation of bacteria or toxins into the blood stream, as in Kitt's method of vaccination against symptomatic anthrax and in immunization with rattlesnake venom.

2. Injection of attenuated virus or toxin. Attenuation may be accomplished by air and light (chicken-cholera, Pasteur) ; by cultivation at high temperatures (anthrax, Pasteur) ; by chemical agents (anthrax, Roux; diphtheria and tetanus toxins, Behring and Roux) ; by desiccation (rabies, Pasteur) ; by passing the virus through other animals (swine erysipelas, Pasteur). This last observation was a most instructive one; passing the bacillus through the rabbit several times increased its virulence for the rabbit but decreased it for swine, while passing the organism through the dove increased its virulence for swine.

3. Injection of killed organisms (anthrax, Toussaint; swine plague, Salmon and Smith). This is the safest means of vaccinating against cholera, typhoid and plague. In the Pasteur treatment of hydrophobia the first injection of the dried spinal cord probably contains the killed virus.

4. Injection of bacterial constituents (*a*) Bacterial cell plasm (Buchner's plasmin, obtained by submitting micro-organisms to high pressure, and Koch's tuberculin TR) ; (*b*) Soluble bacterial

products (the bacterial proteins, as Koch's old tuberclin and mallein; the soluble toxins; products of bacterial autolysis). When toxins are injected antitoxins are formed. The autolytic products of some organisms, e. g., typhoid and dysentery, cause the formation of bactericidal amboceptors and agglutinins, but not antitoxins.

B. Passive immunization: the prophylactic injection of antibacterial and antitoxic serums.

C. Mixed active and passive immunization: the simultaneous injection of an immune serum with the corresponding organism, which may be killed or living. The serum causes immediate, though temporary, resistance, and, in the meantime, an active, more permanent immunity develops as a consequence of the injection of the organisms. This method has been practiced with swine plague, swine erysipelas, rinderpest, and experimentally in typhoid, cholera and plague.

II. CURATIVE INJECTIONS.

A. Active immunization.

1. Injection of killed micro-organisms in small doses with the intention of hastening antibody formation, as suggested by Fraenkel in the treatment of typhoid fever; value not yet demonstrated.

B. Passive immunization.

1. With antitoxic serums: diphtheria, tetanus, snake bites, plague, tuberculosis (?), typhoid (?), streptococcus infections (?), etc.

2. With antibacterial serums: typhoid, cholera, plague, dysentery, streptococcus (?), staphylococcus (?) and pneumococcus (?) infections.

In general, serums to be effective must have a

General Strength of Serums.

certain strength. When diphtheria antitoxin was first used preparations were put on the market which contained twenty or fewer antitoxin units per cubic centimeter, a strength which would necessitate the injection of 150 c.c. or more in order to introduce 3,000 units. Much of the early criticism of diphtheria antitoxin is traceable to the low value of the serums used at that time rather than to an injurious effect on the patients. If diphtheria antitoxin now contains less than 250 units per c.c. it is considered unfit for use; many serums contain 500 or more units per c.c.

Antitoxic and other serums should be free from micro-organisms and toxins. The cases of tetanus which developed in St. Louis following the injection of diphtheria antitoxin will be remembered. With correct governmental supervision of the manufacture of serums, such accidents are entirely preventable.[1]

For the sake of simplicity we may consider the principles involved in serum therapy under the three topics of (*a*) antitoxins, (*b*) bactericidal or antibacterial serums, and (*c*) vaccination.

(A) ANTITOXINS.

Antitoxins.

It has been sufficiently emphasized that neutralization of toxin by antitoxin implies a chemical union between the two substances. When the two are mixed outside the body at a given temperature and at a given concentration, the rapidity and completeness with which the union occurs depends only on the degree of affinity which one has for the other. There is no third substance with which one or the other may unite. In the body, however, the

1. See appendix to Chapter VII (Part I).

conditions are more complex; in this case two combinations are possible for the toxin, one with the antitoxin which has been introduced and a second with the tissue cells. As an instance of the great rapidity with which toxin may unite with cells, the work of Heymans with tetanus toxin may be cited. "Heymans found that, if all the blood were removed from an animal a few minutes after the injection of a single fatal dose of tetanus toxin and the blood of another animal substituted, still the animal died of tetanus" (Ritchie); that is to say, all the toxin had been bound by the cells in that brief time.

Binding of Toxin by Tissues.

Other experiments show that quantities of toxin and antitoxin which are neutral when mixed before injection are not entirely neutral if injected separately and at different points of the body. In this instance some of the toxin has had time to unite with tissue cells before it could come in contact with the antitoxin.

Certain work by Dönitz illustrates not only the rapidity with which toxin may be bound by the tissue, but also the method by which antitoxin effects a cure. In relation to tetanus he found that if the toxin were injected first and the antitoxin four minutes later, a quantity of antitoxin, which was slightly in excess of the neutralizing dose, was required to prevent the development of tetanic symptoms; if he waited eight minutes, six times as much antitoxin; after sixteen minutes, twelve times as much; after one hour, twenty-four times the simple neutralizing dose was required. A few hours later no amount of antitoxin could save the animal. Similar conditions were met in the neutralization of diphtheria toxin by its antitoxin in

the body. Madsen, in performing what he called "Curative Experiments in the Reagent Glass," found that the longer tetanolysin had been in contact with erythrocytes, the more antitetanolysin was required to tear away the toxin from the corpuscles. Practical experience with diphtheria also indicates that the longer the disease lasts the more antitoxin is required for cure.

Nature of Curative Action.

The experiments just cited give us a clear conception as to what is meant by the curative action of an antitoxin—an action which consists not of the neutralization of the circulating toxin, but of the wresting away from the tissue of the toxin which has been bound. Incidentally the circulating toxin is neutralized, and for this step, which is essentially prophylactic in nature, the simple equivalent of antitoxin is required. But for the wresting of toxin from tissue cells not a mere equivalent of antitoxin, but a great excess, is required, as shown by the experiments of Dönitz and of Madsen.

When diphtheria or tetanus has advanced so far that no amount of antitoxin will effect a cure, the relation of the toxin to the cells has become something more than mere chemical union. Further processes of a biologic or biochemic nature have set in in which the toxin may have become an integral part of the protoplasm, and the toxophorous group may have begun its destructive action, whatever the nature of this action may be.

It is important to recognize that antitoxin can not repair an injury already done by the toxin. The repair of the injury depends on the recuperative power of the cells; hence, antitoxin cures by tearing from the cells, perhaps not all, but so much

of the toxin that less than a fatal dose remains in the cell.

Two Important Principles. We may learn from the experiments of Dönitz and of Madsen two important principles of antitoxic therapy: First, that of early administration, i. e., before a fatal amount of toxin has been bound, and, second, the necessity of injecting sufficient quantities of antitoxin.

The comparative study of diphtheria and tetanus has clarified the principles of antitoxic therapy to no small degree. Knowing that diphtheria antitoxin has a much greater curative value than tetanus antitoxin, we find some conditions which would seem to explain the difference, at least in part.

Tetanus. In regard to tetanus we have the following facts: In the test-glass the affinity between the toxin and antitoxin is rather weak, since approximately forty minutes are required for complete neutralization (Ehrlich). On the other hand, the experiments of Dönitz and of Heymans show that the affinity of the toxin for nervous tissue is exceedingly strong, all the toxin being taken up within a few minutes. These two conditions alone suggest the probability of a low curative value on the part of the serum. The toxin of tetanus also has a remarkable selective action on the most vital of all organs, the central nervous system; hence, a lower grade of injury may prove fatal than in other infections in which less important organs or those of greater recuperative power are involved chiefly. Furthermore, it seems (Meyer and Ransom, Marie and Morax) that the tetanus toxin is taken up by the nerve endings and reaches the ganglionic cells by way of the axis cylinders,

whereas the antitoxin which is injected remains chiefly in the blood and lymphatic circulations. Hence, the toxin, to a certain extent, is isolated and less accessible to the action of the antitoxin.

Diphtheria.

Concerning diphtheria, the affinity between toxin and antitoxin is relatively strong, for complete neutralization in the test-glass takes place in about fifteen minutes (Ehrlich). On the other hand, clinical experience indicates that the affinity of diphtheria toxin for tissue cells is less than that of tetanus toxin, for diphtheria may readily be cured on the second or third day of the disease, whereas a cure of tetanus is rarely affected. These would seem to be favorable conditions for successful serum therapy. Although the toxin of diphtheria may attack the nervous system, the paralysis seen in such cases is seldom fatal. On the basis of anatomic findings in fatal cases it seems probable that the greater portion of the toxin is taken up by parenchymatous and lymphatic organs, and by connective tissues (animal experiments), which compared with the nervous tissue are of less immediate importance for life and have greater recuperative powers. We may infer from clinical experience that diphtheria toxin is so situated in the body that it is accessible to the action of the antitoxin.

Important Conditions for Success.

We have, therefore, the following factors which apparently are of importance for the success of antitoxic therapy: 1. The concentration (strength) of the antitoxin which is injected. 2. Its freedom from contamination and adventitious toxins. 3. The time of its administration. 4. The quantity injected. 5. The degree of affinity between toxin and antitoxin. 6. The degree of affinity

between toxin and tissue cells. 7. The amount of toxin which may be bound without a fatal issue, of which the vital importance of the organs involved and their recuperative powers are factors. 8. The location of the toxin in the body, i. e., its accessibility for the antitoxin.

Prophylactic Action of Antitoxin.

What has been said relates to the curative action of antitoxin. It is evident that the action of antitoxin, when used as a prophylactic, is of a simpler nature, for in this instance the conditions approximate those of the test-tube experiment. There has been opportunity for the antitoxin to become uniformly distributed in the blood and lymphatic circulations; hence, it is able to meet and to bind the toxin before the latter comes in contact with the receptors of important cells. The high value of tetanus antitoxin as a prophylactic, a value which has become evident in recent years, probably depends on this condition.

The immunity which is afforded by a prophylactic injection of antitoxin is of short duration, from two to three weeks; the antitoxin is excreted in the urine to a considerable extent, but in part may be bound and assimilated by the tissues.

(B) BACTERICIDAL OR ANTIBACTERICIDAL SERUMS.

Bactericidal Serums.

Attention has been directed repeatedly to a large group of organisms the toxic constituents of which are integrally associated with the protoplasm of the microbes; the toxic substances are endotoxins. Certain members of this group, of which the typhoid, paratyphoid, colon and dysentery bacilli and the vibrio of cholera are representatives, cause the development of strong bactericidal serums in the immunized animal. In Chapter

XII, A, it was shown that such serums have no power of neutralizing the endotoxins of the corresponding organisms; hence, whatever prophylactic and curative properties they may have would seem to depend on the bactericidal action of the amboceptor-complement complex. As to whether the substances which stimulate phagocytosis, i. e., the opsonic or bacteriotropic substances are of importance for the *intra vitam* action of bactericidal serums, remains to be definitely established.

Curative and Prophylactic Power.

It is common knowledge that bactericidal serums have not been successful curative agents, although in test-glass experiments they may be able to kill large numbers of organisms. Experimental work has brought to light a number of conditions which render their ineffectiveness somewhat intelligible, but this knowledge has been of little service in increasing their value, and at this moment their outlook as curative agents is not very encouraging.

Animal experiments indicate that, prophylactically, they are much more powerful than when used as curative agents. Unfortunately, however, as in the case of antitoxins, the immunity which is conferred is of short duration, the serum being excreted or the antibodies destroyed within two or three weeks. For this reason they are not suited for general prophylactic use in man, but they may be distinctly useful when combined with vaccination, as indicated later.

Time of Injection.

Bactericidal serums are efficient in saving experiment animals, provided the serum is injected in advance of, simultaneously with or very shortly after the bacteria are introduced. By injecting the vibrio of cholera and anticholera serum simul-

taneously one may readily save a guinea-pig from ten times the fatal dose, or more. If the culture be injected first and the serum later a larger amount of serum is required to save the animal. After a few hours a sufficient amount of serum to kill all the vibrios may be injected, yet the animal will die from the action of the endotoxins which have been liberated. The organisms had proliferated to such an extent that the mass, though dead, contained a fatal amount of endotoxin. A statement made previously may be repeated, that the administration of a bactericidal serum rather than being beneficial may actually be injurious, in that it dissolves the micro-organisms rapidly, an excessive amount of endotoxin thereby liberating; this, perhaps, is not definitely established as a point of practical importance.

Having determined the amount of a bactericidal serum which is able to save a guinea-pig from an incipient infection, one may calculate on the basis of weight the amount which would be required to save a man under the same conditions; frequently it amounts to impossible quantities, hundreds of cubic centimeters. The conditions are all the less promising when we remember that physicians are usually called on to treat well-established rather than incipient infections.

Peculiarities of Complement and Amboceptors.

Other conditions which operate against the effectiveness of bactericidal serums as curative agents have to do with peculiarities of complements and amboceptors. The lability of complement involves certain difficulties. A bactericidal serum, as one would purchase it, contains none, because of its spontaneous degeneration. Theoretically, this difficulty may be obviated in three

ways: First, one may use serums which are fresh from the immunized animal; second, one may complement the solution of amboceptors (old immune serum) by the addition of fresh serum from a normal animal which is known to contain suitable complement; or, third, one may inject the complement-free serum and place reliance on the complement which exists in the plasma and lymph of the patient for activation of the amboceptors. It is sufficiently established that none of these procedures enhances the curative value of the serums to a satisfactory extent.

Absorption of Complement by the Tissues.

Regardless of the amount of foreign complement which is introduced, it appears to be diverted from its function. It has been shown experimentally that the tissues may absorb a foreign complement, and the mere fact that anticomplements are formed so readily indicates that complement may be bound by the tissues. In accordance with a rather general principle, if the animal which furnishes the serum is remote from man zoölogically there is all the more likelihood of the complement being fixed by human tissues.

Choice of Animals for Immunization.

It has been suggested that if one should choose for immunization animals which are closely related to man, as chimpanzees and monkeys, a double advantage would be gained: First, the foreign complement may be identical or similar to that in man and consequently would be less likely to be absorbed by the tissues; and, second, the complementophilous haptophores of the amboceptors may be so constructed that human complement would serve for activation. Theoretically, the conditions would be ideal if immune human serum were available for therapeutic purposes.

If one depends on the complement in the patient's body for activation of the amboceptors, there are two possible difficulties of importance: First, the native complement of the body is often decreased during infections and in some chronic diseases and may be too little for thorough activation; second, the amboceptors of the immune serum may demand for their activation a complement or complements which the body does not contain.

Diversion of Complement.

Diversion of complement has been referred to as a phenomenon seen in test-tube experiments. In this condition an excess of amboceptors in some way decreases the power of the serum; by an excess of amboceptors one means, in this instance, such a quantity that many are unbound by the bacteria. It is supposed that a certain amount of the complement is absorbed by free or unbound amboceptors, hence the effect is like that of too little complement. In the desire to administer a sufficient amount of antibodies, so much may be introduced that diversion of the complement occurs in the body. Results obtained by Löffler and Able, by Pfeiffer and by Buxton and others, in which excessive doses of immune serum were less protective than moderate doses, show that a similar phenomenon occurs in the body.

Inaccessibility of Microbes.

In certain diseases the microbes are so situated that a serum as ordinarily administered may not be able to reach them. Pfeiffer thinks that there is little hope for the serum treatment of cholera because of the exclusive location of the living organisms in the intestinal tract. In typhoid also the intestines are a reservoir of typhoid bacilli, although the living organisms reach the circulation in abundance.

By way of summary, the following conditions appear as factors in the low curative value of bactericidal serums: 1. Bactericidal serums are not antitoxic. 2. They may liberate an excessive amount of endotoxin by dissolving the bacteria. 3. The lability of exogenous complement. 4. The power of the tissues to absorb the complements of a foreign serum. 5. The lack of a sufficient amount of suitable complement in the human body. 6. The difficulty of obtaining amboceptors for which human complements are suited. 7. The possibility of diversion of complement by an excess of amboceptors. 8. Inaccessibility of the micro-organisms in certain infections (cholera, typhoid).

Other "Antibacterial" Serums.

As pointed out elsewhere, another group of organisms, the members of which contain endotoxins, causes the formation neither of antitoxins nor of bactericidal serums; streptococcus, staphylococcus, pneumococcus, etc. Many investigators, nevertheless, are positive in their claims that the antiserums for these organisms have a protective and even a curative value. The properties on which their value depends have not been satisfactorily ascertained. Although certain antistreptococcus serums are said to be antitoxic, it is contended by others that they act by simulating phagocytosis. Work now in progress promises to show that immunization with these organisms causes an increase in the opsonins. Their curative value is very low in experimental work and they fail totally if injected a few hours subsequent to the introduction of the organisms. Clinically, we are familiar with them as failures.

It is particularly in relation to the streptococ-

cus that the so-called polyvalent serums have been prepared. Cultures of streptococcus obtained from numerous sources are used in the immunization with the expectation that the serum will be effective against various strains of streptococci. The principle may be an important one in the preparation of other antibacterial and bactericidal serums.

(c) VACCINATION.

Vaccination or Protective Inoculation.

We are most familiar with the terms vaccine and vaccination as applied to protective inoculation against smallpox. They are used, however, with equal propriety in all instances in which the attenuated or killed virus of a disease is inoculated for the purpose of establishing resistance to an infection. The process set in motion by vaccination is one of active immunization in which the cells are induced to form specific antibodies over a long period; hence, the resistance is more protracted than that established by passive immunization.

Certain experimental work, as previously stated, indicates that the acquired resistance persists after the formation of antibodies has ceased, even after the quantity of the latter has sunk to the normal. This condition has been explained by assuming that, as a consequence of vaccination, the cells of the body have been "trained" to produce the corresponding receptors; hence, when the micro-organisms gain entrance at a subsequent time new antibodies are formed so rapidly and in such abundance that the incipient infection is overcome.

In some instances the nature of the virus used is unknown, as in smallpox and hydrophobia; in all probability, however, it consists of micro-or-

ganisms rather than of toxins alone. In the case of typhoid, cholera, plague and other diseases of known etiology pure cultures, living or killed, are inoculated. Protection does not follow immediately on the inoculation. We are sufficiently familiar with this fact in relation to smallpox, in which several days are required for the formation of a protective amount of the antibodies. There is reason to believe that the interval between the inoculation and the appearance of antibodies is characterized by a decreased resistance on the part of the individual, so that during this brief period he is unusually susceptible to infection.

Negative and Positive Phases.

That period immediately following the injection of a toxin or microbe, in which the quantity of antibodies undergoes a temporary decrease, Wright speaks of as the negative phase of the immunization; whereas that period marked by the new formation of antibodies is called the positive phase. The negative phase lasts from a day or two to several days, depending on the quantity and nature of the virus injected (typhoid). A second injection should not be given during the negative phase, since it causes a further decrease in the antibodies and prolongs the phase. Wright speaks of this as a cumulative negative phase. A cumulative positive phase, marked by the formation of larger amounts of antibodies, may be induced by the proper spacing of a number of injections.

Nature of Antibodies.

In certain instances the nature of the antibodies is known. In typhoid, cholera, plague and dysentery, for example, they consist of bactericidal amboceptors; agglutinins and precipitins are formed incidentally. The amboceptors naturally depend on the complement of the body for their

activation. If the disease is one of unknown etiology the nature of the antibodies is not easily determined. We should keep in mind the possibility that vaccination may cause an increase of the opsonins and that the potential phagocytosis may thereby become greater.

In case the incubation period of the vaccination is shorter than that of the disease (smallpox, hydrophobia) vaccination usually is successful even if practiced within a limited time after exposure to infection.

Vaccination in individual diseases is considered in Part II (see also Chapter VI, pp. 57, 58).

Theoretically it would be possible to immunize man against diphtheria and tetanus by inoculating with small amounts of the corresponding toxins. Such a procedure, for obvious reasons, would be unnecessary and unjustifiable.

Mixed Active and Passive Immunization.

It is not unlikely that mixed active and passive immunization will be of great service in some infections. A successful campaign against rinderpest has been carried on in the Philippines by this method. The blood of infected cattle contains the virus, which as yet has not been cultivated artificially. The serum of cattle which have recovered from the disease, or which have been immunized cautiously with infected blood, contains the specific antibodies. Both the immune serum and virulent blood are used for the inoculations. The same principle has been found effective in experimental work with cholera, typhoid and plague. Immediate immunity is established by the serum, which would eliminate the danger period mentioned above, and before the serum disappears entirely active immunity develops.

PART TWO—SPECIAL.

Although a consistent classification of the infectious diseases, on the basis of immunity, is impossible at the present time, a certain grouping is desirable for the sake of convenience. The following arrangement of those diseases we are able to consider is made on a basis which is partly etiologic, partly with reference to the pathogenic properties of the micro-organisms, and partly to the nature of the reactions excited in the body by infection or immunization. In some instances nothing more than general analogies suggest themselves as a basis for the grouping, which is necessarily provisional and imperfect.

GROUP 1.

Diseases, natural or experimental, which are caused by soluble toxins of bacterial, animal or plant origin. Infection or immunization induces immunity to subsequent attacks (except in hay fever), the immunity being characterized by the formation of serum antitoxins, and occasionally of bacteriolysins and agglutinins. The serums of highly immunized animals are protective and curative for the corresponding intoxications in man and other animals.

A. BACTERIAL DISEASES.

I. DIPHTHERIA.

Bacillus diphtheriæ, or the Klebs-Loeffler bacillus, was discovered by Klebs in 1883, and more fully described by Loeffler in 1884. It answers all

Characteristics of the Organism. Koch's laws in its relationship to the disease of diphtheria. It is a non-motile, rod-shaped organism having about the length of the tubercle bacillus, but twice its thickness. One end commonly presents a flask-like enlargement. It stains by Gram's method, with the ordinary anilin dyes, and with the special stain of Neisser shows a peculiar granulation, the granules of Babes-Ernst. It is readily cultivated, especially on solid media which contain serum and in various bouillons. It tends to grow in coherent masses and under the microscope the cells often show a characteristic phalanx-like arrangement.

The diphtheria bacillus is an obligate parasite having no vegetative existence outside of the body, is very resistant to desiccation and may remain virulent in a dried state for from one to five months. Its life in water varies from a few days to several weeks, having its shortest existence in distilled water and its longest in hydrant water which has been boiled. It disappears more quickly from unboiled hydrant water. It is very susceptible to ordinary antiseptics, being killed in a few minutes by corrosive sublimate even in a dilution of 1 to 10,000.

Methods of Infection. The sources of infection may be enumerated as follows: 1. From the false membranes, sputum or excretions of the mouth, pharynx, nose, conjunctiva and deeper respiratory passages of infected individuals. 2. From convalescents and those who have fully recovered, even after serum treatment. Virulent organisms may persist in the pharynx or nose of convalescents for weeks and months, as in one of Prip's cases in which they were found twenty-two months after recovery. 3.

From the upper air passages of healthy persons who may never have had diphtheria, but who have been in direct or indirect contact with the diseased. Kober obtained virulent bacilli from 8 per cent. of the individuals who had been in direct contact with patients, and he states that 0.83 per cent. of the people at large carry with them virulent organisms. This condition may well account for the "spontaneous" origin of diphtheria in the susceptible. 4. From cases of latent diphtheria as represented by chronic pharyngeal diphtheria and chronic *rhinitis fibrinosa.*

Hence, infection takes place chiefly by direct contact, but frequently also by indirect contact. Transmission by kissing or by other means of intimate contact, by using infected cups or toys, is well recognized. "Drop infection," i. e., from infected globules of mucus or saliva which the patient emits when speaking or coughing, may occur, but perhaps is not of great significance. The same probably is true of "dust infection," although, as stated, the organism may remain living and virulent in a dried state for a long time. The disease is rarely transmitted from animals to man, although such transmission may occur from the cat, which occasionally suffers from true diphtheria. The diphtheria of fowls is due to another organism.

The upper air passages, more rarely the conjunctiva, wounds and the vulva, are recognized as infection *atria.*

Pathogenesis.

The local and general phenomena of diphtheria are caused by the soluble toxin which the organism secretes. Although the toxin is not absorbed through, nor does it injure the unbroken skin, it

produces necrosis of the mucous surfaces and underlying tissue at the site of infection. Through the wounded surface fibrin-forming elements escape, as a consequence of which successive layers of fibrin are deposited and the fibrin, together with the necrotic surface, leucocytes and associated micro-organisms constitute the membrane which so often marks the disease clinically. The local process is similar in diphtheria of cutaneous wounds. The toxin becomes generalized by absorption through the lymphatic circulation.

Localization of the Bacilli.

Characteristically the bacilli are confined to the site of infection. Although diphtheritic bacteriemia rarely occurs, the bacilli have been found occasionally in the blood and viscera of fatal cases.

The clinical and anatomic conditions lead us to believe that the parenchymatous organs, the lymphatic tissues and the cells of the nervous system contain receptors with which the toxin unites, inasmuch as these tissues suffer demonstrable injury during the disease. When the toxin is injected subcutaneously into animals, localized edema and necrosis occur; hence, the connective tissues may also take up a portion of the toxin, diverting it, so to say, from the more vital organs.

Mixed Infections.

Mixed infections render diphtheria a more dangerous disease. According to Baumgarten, the streptococcus is associated with the diphtheria bacillus in most cases of diphtheria. The observation of Roux and Yersin that the streptococcus increases the virulence of the diphtheria bacillus both in the test-tube and in animal experiments may explain to some degree the severity of the disease when accompanied by streptococcus infection. Aside from the local influence of the streptococcus,

however, a general invasion by this organism may occur, with such consequences as acute nephritis or lobular pneumonia, and in this condition the diphtheritic infection may fall into the background in importance (septic diphtheria). Post-diphtheritic suppurations commonly are caused by the pyogenic cocci, but sometimes in association with the diphtheria bacillus itself. Rarely the bacillus is found in pure culture in lobular pneumonia, a condition which Flexner and Anderson produced experimentally in animals. In puerperal infections with the streptococcus a puerperal diphtheria is sometimes superimposed.

Immunity and Susceptibility.

Very young children resist diphtheritic infection. A certain degree of immunity may be transmitted by the mother. Observations on animals show that when the blood and milk of the mother contain antitoxin, the offspring acquires some protection, which, however, may disappear after the cessation of nursing. Polano claims that antitoxin passes from the mother to the child through the placenta. From the second to the seventh or eighth year children usually are very susceptible. This susceptibility is not uniform, however, for many children escape infection, whereas others, under the same conditions, contract the disease. Following this period susceptibility decreases and after the fifteenth year the disease is relatively rare.

The cause of the immunity which develops in the absence of a preceding infection has not been sufficiently investigated. In some cases considerable amounts of antitoxin are found in the serum, perhaps enough to account for the immunity. The prolonged presence of bacilli of low

virulence in the nose or pharynx, or mild attacks of the disease which have not been recognized, may cause the development of antitoxin. As stated in an earlier chapter, the loss of suitable receptors may be a factor in this type of acquired immunity.

Hypertrophic tonsils and chronic pharyngitis appear to be predisposing causes in children.

Active Immunity.

Spontaneous recovery (active immunity) is due solely to the formation of the specific antitoxin by the tissues of the patient. We may regard the relationship of the leucocytes to diphtheritic infection as not definitely settled. Although leucocytosis is a fairly constant occurrence and may go as high as 25,000 to 30,000 to the cubic millimeter, it is difficult to dissociate that due to the diphtheritic infection from that caused by a mixed infection with the streptococcus. Both polynuclears and mononuclears are increased, the latter being especially marked in children (Ewing). That the polymorphonuclear leucocytes may ingest diphtheria bacilli was shown by Wright and Douglass, the influence of opsonins being essential for phagocytosis. Certain observers hold that a marked leucocytosis is an unfavorable prognostic sign, although Besredka and others take the opposite view.

The duration of active immunity to diphtheria varies greatly. Usually an individual has diphtheria but once, yet not infrequently those are encountered who suffer from repeated attacks. In some instances the susceptibility continues into adult life.

Prophylaxis.

The advent of serum therapy justifies no relaxation in the customary prophylactic measures, such

as isolation of the diseased, quarantine and disinfection. A patient should not be considered harmless until his mouth, pharynx and nose are free from bacilli, a condition which may be brought about by antiseptic applications, and for the determination of which repeated bacteriologic examinations are necessary. The danger that others who have been in contact with the patient may carry the infection should be met by appropriate treatment. It is not to be forgotten that antitoxin does not destroy the organisms. The injection of antitoxin is our most effective measure for individual prophylaxis.

Serum Therapy.

The efficacy of diphtheria antitoxin is so well known that little comment is needed. It has caused a reduction of more than 50 per cent. in the mortality of the disease; from 41 per cent. to 8 or 9 per cent., according to Baginsky.

For prophylaxis from 500 to 1,000 units are generally recommended, although some foreign authorities give only 250 units. Rarely, individuals who have received such treatment develop diphtheria within twenty-four hours after the injection. In these cases it is probable that infection has already occurred and symptoms appear before the antitoxin is thoroughly distributed. Naturally one may contract diphtheria after the antitoxin is eliminated.

For curative purposes the amount actually required depends on the virulence of the infection and the duration of the disease. Inasmuch as the virulence may not be known accurately, what appears to be an excess of antitoxin is always demanded. Having in mind the average dose of 3,000 units recommended by the recent edition of

the United States Pharmacopeia, the physician must be guided by the conditions in the individual case. Less than 2,000 units are rarely indicated, and as many as 10,000 and 14,000 units may be given without detriment to the patient. There should be no hesitation about repeating a dose within twenty-four hours in the absence of distinct improvement.

Ransom and Knorr state that if the antitoxin is given intravenously, which may be done without danger, the action of the serum is about eight hours earlier than when given subcutaneously. In severe and in late cases it is advisable to use this method of introduction, the serum first being warmed to the temperature of the body.

It is probable that few cases are so mild or so hopeless, unless moribund, that the omission of antitoxin is justifiable.

Diphtheritic Paralysis.

The belief that antitoxin favors the development of diphtheritic paralysis is no longer held. If there has been an actual increase in the percentage of cases which suffer from paralysis, as sometimes stated, it is because a larger number of severe cases is saved; and the severe cases are those which most frequently develop paralysis. If we accept the view of Ehrlich that a special toxin of weak affinity for the antitoxin, i. e., the toxon, causes the paralysis, we find all the more justification for large doses of antitoxin, for antitoxin neutralizes the toxon as well as the toxin. On the basis of experimental work Ransom concludes: "Transferring the results (of experiments) to practice among human beings, we may expect liberal doses of antitoxin given early in the illness to influence favorably the subsequent paralysis; and this fa-

vorable influence is likely to manifest itself, not so much in the local paralyses (soft palate, etc.), as in such fatal symptoms as failure of the heart. Severe cases, however, are likely to be followed by some paralysis in spite of even large doses of antitoxin."

Cases in which there is severe mixed infection, septic diphtheria, respond less favorably to antitoxic therapy than uncomplicated cases. At some time a mixed serum therapy suited to the mixed infection may be possible.

The suggestion made by Wasserman of a combined treatment with bactericidal and antitoxic serums has not been applied practically.

Agglutination Test.

Inasmuch as the serum of the patient does not develop agglutinins, the agglutination test is of no value for the recognition of the disease. If animals are immunized with the bacillus, agglutinins are said to be formed. The serum of such an animal may be used for the identification of a culture made from the throat, but this would have no practical value, for the diagnosis may be established by the ordinary bacteriologic methods much more quickly and satisfactorily. It is difficult to obtain a homogeneous suspension of the bacillus for the agglutination test.

Pseudodiphtheria Bacilli.

Microscopically and culturally the bacillus of diphtheria can be distinguished with difficulty from a variety of other organisms which belong to the same group, and which are called pseudodiphtheria bacilli. The latter are frequently found in diphtheritic throats, but occur also in the upper air passages and conjunctiva in the absence of all lesions. On the whole, they are non-pathogenic, but occasionally a culture is found which causes a

subcutaneous infiltration at the point of injection in an experimental animal. Hamilton cultivated one which was distinctly virulent for animals. Their pathogenicity, however, is altogether different from that of the diphtheria bacillus inasmuch as diphtheria antitoxin does not protect against them nor do animals which are immunized with pseudo-diphtheria bacilli become immune to the toxin of diphtheria. The *Bacillus xerosis,* which is thought by some to be the cause of xerosis conjunctivæ, but which is also found under normal conditions, is a pseudodiphtheria bacillus. The animal experiment is the only positive means of differentiating the true from the pseudodiphtheria bacilli. Some consider them as diphtheria bacilli which have lost their virulence.

The presence of these organisms may complicate the diagnosis of diphtheria in some cases, but there is little danger of serious error. If one found organisms resembling the bacillus of diphtheria in a membranous sore throat which was accompanied by severe symptoms, there could be no wavering in the decision to use antitoxin.

II. TETANUS.

In 1884 Carle and Rattone demonstrated the infectiousness of tetanus by inoculating the pus from an infected wound into rabbits; 11 of the 12 inoculated rabbits died of tetanus. In 1885 the bacillus was discovered by Nicolaier, and Kitasato cultivated it artificially in 1889.

Characteristics of the Micro-organism.

The organism is rather long and slender (2 to 4 microns long, 0.3 to 0.5 broad), possesses many flagella and has a small amount of motility. It stains readily with the ordinary anilin dyes and by

Gram's method. In young cultures isolated cells and threads predominate, but after a few days spore formation begins; eventually all the adult cells degenerate and the culture consists entirely of spores. The spores have a larger diameter than the bacillus, are situated at one end of the cell and give the latter the characteristic "drumstick" form. The organism is a strict anaërobe and is obtained in pure culture with some difficulty. Morphologically it is difficult to distinguish from the bacilli of malignant edema and symptomatic anthrax.

Habitat.

Few organisms are distributed more widely and generously than the bacillus of tetanus. It is most abundant in street dirt and in tilled ground which has been fertilized with manure. Nicolaier found it in twelve out of eighteen samples of earth. It is less abundant in timber land. Such a distribution is easily accounted for, since the bacillus seems normally to be an inhabitant of the intestinal tract of the horse, cow and sheep, and is often found in that of man and other animals. It occurs on dirty clothing and readily gains access to dwellings with dust in which it may be blown and carried about. Tetanus frequently develops in gunshot wounds in which dirty clothing is carried into the tissue, and several instances of house tetanus have been noted in which a number of individuals in the same dwelling have contracted the disease following injury. Particular localities may be heavily infected. In certain tropical districts a large percentage of the new born die of tetanus neonatorum, and puerperal tetanus has prevailed alarmingly in Bombay. It has been suggested that the custom of bleaching the linen on the

ground may be responsible for the prevalence of the disease in these localities, but from the fact that it has decreased under aseptic practices the general lack of surgical precautions is probably of greater importance. Tetanus has resulted from the injection of impure gelatin for hemostatic purposes. The bacillus has been found in sea water.

The ability of the bacillus to proliferate outside the animal body has not been determined. Some observers hold that it exists as a vegetative organism only in the intestinal tract of animals, but the possibility of proliferation in soil is by no means excluded, particularly since it is so often found in association with organisms which are known to favor its growth. When incrusted in solid material and accompanied by suitable saprophytes it may readily find the anaërobic conditions which are demanded for germination of the spores.

Resistance. The spores are very resistant. In one instance they remained virulent for eleven years on a splinter of wood. They may be killed in six days by direct sunlight. In comparison with non-spore-forming organisms they are very resistant to antiseptics. Kitasato found that they were killed in five minutes by steam, in fifteen hours by a 5 per cent. carbolic acid, in two hours by 5 per cent. carbolic acid to which 0.5 per cent. of hydrochloric acid was added, in three hours by 1/1000 corrosive sublimate and in thirty minutes by the same solution to which 0.5 per cent. hydrochloric acid had been added.

Tetanus is conspicuously a wound infection and that it develops so frequently from wounds which are contaminated with earth is readily understood

Infection Atria and Conditions Which Favor Infection.

from the distribution of the organisms as cited above. Considering, however, the great number of such wounds and the prevalence of the bacillus, the rarity of the disease is remarkable. In explanation of this fact investigations have shown that the organism is not a vigorous parasite, that it demands special conditions for its development in the tissues. According to Vaillard and Rouget, the spores when washed free of toxin do not cause tetanus, but rather are taken up and destroyed by leucocytes.

Anaerobic Conditions in Wounds.

The bacillus, furthermore, is a strict anaërobe, demanding for its development a wound from which the air is largely excluded. It is well known that penetrating wounds in which infected material is carried beneath the fasciæ, as the rusty nail wounds, also those accompanied by deep lacerations, as wounds inflicted with blank cartridges, or those in which dirt and micro-organisms have been ground into the tissues, as in crushing injuries, are prone to be followed by tetanus. Under such conditions the bacillus lies deeply imbedded in the tissues and remote from the air.

Inhibition of Phagocytosis.

Of equal importance is the presence of foreign matter and particularly of other micro-organisms. Relatively superficial wounds in which there is laceration of the tissue with consequent necrosis, as in wounds by toy pistols, even the paper-cap pistol, are well adapted for the development of tetanus if the germs were on the skin at the time of injury. Necrotic tissue favors the proliferation of the tetanus bacilli in two ways. In the first place it seals up the wound to a certain extent, and thus provides the requisite anaërobic condition; in the second place it would seem to prevent

phagocytosis of the bacilli in some obscure way. It has been suggested that the strong, chemotactic relation which exists between necrotic material and leucocytes causes the latter to take up the dead tissue rather than the bacilli. That innocent foreign material may favor the development of tetanus in the presence of the microbes was shown by Vaillard and Rouget: tetanus would develop in the presence of an artificially produced hematoma or a subcutaneous fracture while in the absence of such predisposing factors the bacilli were taken up by phagocytes.

Mixed Infections.

Saprophytic organisms and the pus-producing cocci which are usually found in wounds contaminated with earth appear to favor the development of tetanus. This may be explained to some extent by their ability to increase the virulence of the tetanus bacillus, a condition which is noted in cultures. In the wound they may engage the leucocytes in phagocytosis and prevent ingestion of the tetanus bacilli. As aërobic organisms they may facilitate development of the bacilli by consuming local oxygen.

Our great harvest of tetanus following Fourth of July injuries is closely associated in the first place with the warm, dry season in which the bacilli are more readily disseminated with dust, and in the second place with the nature of the wound and mixed infections, as described above.

Occasionally tetanus follows the simplest wounds, which may have healed entirely before symptoms develop. In "idiopathic tetanus" and in the so-called "tetanus rheumaticus," which follows exposure to cold, the infection atria are unknown. In the latter instance a latent infection,

which is stirred into activity by the reduction of resistance which often follows exposure, may be present; avirulent tetanus bacilli (?) were cultivated from the lungs of one such patient. The occasional occurrence of tetanus following diphtheria and typhoid suggests that infection may take place through wounds of mucous surfaces. Neither the bacillus nor its toxins penetrate the unbroken skin or mucous membranes, and the alimentary tract is further protected by the ability of the gastric and pancreatic juices to digest the toxin.

Period of Incubation.

The incubation period varies from two or three days to several weeks. In the statistics of Rose 20 per cent. of the cases showed symptoms in the first week, 45 per cent. in the second, and about 30 per cent. in the third or fourth weeks. The shorter the incubation period the more fatal the disease. In the statistics cited the mortality with short incubation was 91 per cent.; when the incubation period was moderate it was 81.3 per cent., and when prolonged, 52.9 per cent. The nearer the infection atrium is to the central nervous system the shorter is the incubation period; "head tetanus" develops quickly.

Pathogenesis.

The pathogenic properties of the tetanus bacillus reside in its soluble toxins, of which two, tetanospasmin and tetanolysin, are known. The characteristic nervous phenomena of the infection depend on the action of the former, whereas the latter, a hemolytic toxin, is of minor importance. As in diphtheria, a systemic distribution of the bacilli is not necessary for the development of the disease, the toxin being produced by the organisms in the wound, whence it is carried to the nervous tissue by way of the lymphatics. Particularly in

mixed infections tetanus bacilli may be carried to neighboring lymphatic glands and eventually reach the circulation; pure cultures have been obtained from the heart's blood in experimental work. The blood, on account of its content in oxygen, is thought to be unfavorable for the growth of the organism.

Just before death the toxin has been demonstrated in the blood of man by injecting some of the serum into mice. Its excretion in the urine is questionable. Tetanus produces no characteristic anatomic changes, although degenerative lesions in the ganglionic cells occur. Death usually occurs from asphyxia caused by contractions of the diaphragm, or muscles of the glottis, or from cardiac failure. In some instances the blood has been found more or less laked because of the action of the tetanolysin.

Tetanospasmin. Tetanus toxin (tetanospasmin) has a very strong affinity for the nervous tissue of susceptible animals. This may be demonstrated in test-tube experiments in which the toxin is mixed with an emulsion of the nervous tissue; the nervous tissue neutralizes the toxin more or less completely, as determined by subsequent inoculations of the mixture (Wasserman's experiment). It is held by certain authorities that the toxin attacks only the nervous tissue in man; in some of the lower animals, however, various organs, especially the liver, have an affinity for the toxin.

The method by which tetanus toxin reaches the central nervous system has been the subject of much speculation and experimentation. Recent observations by Marie and Morax and by Ransom and Meyer show with a great degree of probabil-

ity that it is absorbed by the end organs of the motor nerves and from there passes to the ganglionic cells through the axis cylinders. This absorption takes place very quickly; when the toxin is given intravenously it disappears from the blood in the course of minutes. It has been found in the nerves within an hour and a half after subcutaneous injection. Its further transmission centrally occupies more time and, indeed, the investigators mentioned explain the rather long incubation period of the disease on the basis of the time required for this transmission. The brief incubation period in "head tetanus," accordingly, would depend on the short distance the toxin is obliged to travel to reach the ganglionic cells.

Varieties of Tetanus.

Although the toxin appears not to be taken up by the sensory nerves, a painful form of the disease, *tetanus dolorosa* (Meyer), may be produced experimentally by injecting the toxin into the posterior roots of the spinal nerves. Roux caused "cerebral tetanus" by introducing the toxin into the cerebral tissue; the condition is characterized by absence of contractures. "Local tetanus," in which the muscles in the vicinity of infection or inoculation are involved in contractures, is the first symptom of tetanus in experiment animals; it rarely occurs in man except in head tetanus. The phenomenon depends on the fact that the toxin, being transmitted through the motor nerves, reaches first the ganglionic cells which correspond to the infected area.

Immunity in Man.

According to Metchnikoff, the only natural immunity which man possesses to tetanus is leucocytic and this may be sufficient to protect under favorable conditions. The observations of Vaillard

and Rouget (cited above) support this claim. Susceptibility depends not only on the presence of suitable receptors in the nervous tissue, but also on the degree of affinity which exists between these receptors and the toxin. In man and some animals this affinity is very great, whereas in fowls it is weak and an enormous amount of toxin is required to cause tetanus. A further proof of this weak affinity in non-susceptible animals rests in the fact that the toxin when injected into the blood remains unabsorbed for a long time, whereas in susceptible animals it disappears very quickly. Acquired immunity depends on the presence of antitoxin in the circulation.

Prophylactic Value of Antitoxin.

Tetanus antitoxin is a thorough prophylactic. This fact has been heralded so extensively in recent years that there can be little excuse for ignorance on the part of any physician. At the same time, the returns from the "Fourth" show that the principle is not yet deeply imbedded in the medical mind. It is quite certain that a large percentage of these fatalities could be prevented by two injections of antitetanic serum, one at the time of injury and a second from five to eight days later. An epidemic of puerperal tetanus in an obstetric ward in Prague was checked by prophylactic injections of the antitoxin. In a certain section of France 4,000 horses, with injuries commonly followed by tetanus, received antitoxin and none developed the disease.

No degree of efficacy on the part of the antitoxin, however, justifies disregard of the surgical care which the wound demands. From the facts cited it is clear that thorough and frequent disinfection of the wound, free drainage, the removal

of all foreign and necrotic material, and the access of air are measures of eminent importance. Punctured wounds should be opened up. Antitoxin, preferably as a powder, may be used in the wound, and the serum infiltrated into the adjacent tissue.

Curative Value of Antitoxin.

The principles which apparently underly the ill success of the antitoxin as a curative agent were treated of in Chapter XVI, Part I. Its administration as early as possible after symptoms have appeared is demanded. After symptoms have existed for more than thirty hours Behring maintains that there is no hope of cure by the subcutaneous route. Inasmuch as forty hours or more are required for complete absorption from the subcutaneous tissue, intravascular injection of at least the first dose would seem to be indicated. Yet by neither of these methods is the most essential end accomplished, for the antitoxin does not reach the nerves nor can it be recognized in the cerebrospinal fluid in conspicuous quantities. The most that such injections accomplish is the neutralization of the circulating toxin, that which is not yet on its way to the central nervous system through the motor nerves. It is, of course, important to neutralize the circulating toxin and it must be done quickly, for in the course of a few hours the fatal quantity of toxin may have been absorbed; "a dose of antitoxin which would save in the morning may be without effect in the evening."

Method of Using the Antitoxin.

At the same time it is of greater immediate importance to neutralize that which has already entered the peripheral nerves, and if possible to tear away some of the toxin already bound by the ganglionic cells. To accomplish this object, or to at-

tempt it, special procedures are demanded. We may then consider the antitoxic treatment as follows:

First: The neutralization of the toxin which has already been absorbed by the peripheral nerves and spinal cord at a point as near the vital centers as possible. This involves surgical exposure of the large nerves of the part as near the trunk as possible and their infiltration with antitoxin (Ransom and Meyer), and in desperate cases the infiltration of the antitoxin in the spinal cord in the vicinity of the medullary centers. From five to fifteen minims may be injected into the nerve trunks at a sitting, and the operation may be repeated on subsequent days; the needle should be partially withdrawn and reinserted in different directions during the injection. Rogers recommends tying loose ligatures around the nerves after the operation so that they may be readily drawn up and identified for further injections. In order to reach the medulla the intracerebral method of Roux or that of Rogers may be utilized. Kocher has devised a technic for the intracerebral injections. Anterior to the parieto-frontal suture and to one side of the median line the scalp is prepared, and a hole drilled through the skin and skull, having its direction toward the foramen magnum. By means of a long needle, the ventricle is penetrated and the serum, after injection, finds its way to the fourth ventricle to the imperiled respiratory and cardiac centers; 10 c.c. may be injected. Rogers seeks to accomplish the same end by a different technic. He introduces the needle between the sixth and seventh cervical vertebræ, punctures the cord deeply, and injects from

20 to 30 minims at a sitting. Although there is danger of intraspinal hemorrhage in the procedure, no ill effects were noted. It has been recommended also that the cerebrospinal fluid be withdrawn by means of lumbar puncture and substituted by antitoxin. Some physicians who have used this method report favorable results.

Second: The neutralization of all toxin which is not yet bound by the nervous tissue or absorbed by the motor nerves. This demands the infiltration of the wound and surrounding tissue with the antitoxin, and injection of a sufficient amount of the serum into the circulation in order that circulating toxin may be neutralized. The intraneural, intraspinal or intracerebral injections should always be supplemented by subcutaneous or intravascular injections. The first dose should be given intravenously, whereas subsequent injections may be given subcutaneously. The injections should always be repeated.

Standardization of Antitoxin.

Unfortunately, tetanus antitoxin is not standardized by American manufacturers and dosage can not be controlled with any accuracy. Although standardization can not be accomplished with the same degree of accuracy as in the case of diphtheria antitoxin, its approximate value can be determined (within 5 or 6 per cent.), which is sufficient for practical purposes. The antitetanic serums of Behring, Tizzoni and the Pasteur Institute are all standardized, but on somewhat different bases. Behring advises the administration of 20 units of his serum for prophylactic purposes, and 100 units as the "simple" curative dose when given soon after the development of symptoms.

Not less than 10 c.c. of American serum should

be given for prophylaxis, and the dose should be repeated. No definite limits can be given as to the amount which may reasonably be given for curative purposes. Ten cubic centimeters given intravenously at once, and an equal amount subcutaneously on subsequent days, would seem to be sufficient to neutralize the unbound toxin if the serum has reasonable strength. Standardized serums certainly are to be preferred.

Agglutination has no practical significance for diagnostic purposes. An agglutinating power has been noted in the serum on the eighth day. Agglutinins may be produced by immunizing animals (rabbits) either with the bacilli or the toxin. In the latter case the formation of the agglutinin is due to the presence of agglutinogenic receptors in the toxin solution.

III. BOTULISM.

Bacillus Botulinus.

Botulism is a peculiar form of meat poisoning in which the nervous system is involved principally. From twenty-four to thirty-six hours after the poisonous meat is eaten salivation, ptosis, dilatation of the pupils and paralysis of the ocular muscles develop and death from bulbar paralysis occurs rapidly in from 25 to 30 per cent. of the cases. In the event of recovery, convalescence may extend over weeks or months.

Infected Meats.

The disease occurs especially in some European districts in which improperly preserved or raw meats are eaten. The term ichthyosismus is applied to a similar or identical disease which is caused in Russia by salted fish. In 1895 von Ermengem investigated a ham which had caused 50 cases of botulism, and isolated from it an anaërobic, spore-forming bacillus, which produces a

soluble toxin capable of causing the entire symptom-complex of the disease.[1] The organism possesses flagellæ, has limited motility, grows only in alkaline media, and in contrast to most pathogenic organisms prefers a relatively low temperature (18-25° C.). It is probably on account of its physiologic activity at such temperatures that it is able to produce its toxin in meats which have been kept in a cool place. It is found in decomposed ham and various sausages (Leberwurst and Blutwurst), and probably gains access to the meat after the animal has been killed. Von Ermengem investigated two hams from the same animal. One was under anaërobic conditions being covered with brine, while the other was exposed to air; only the former was toxic. The organisms may be absent from the superficial portion of the meat, but abundant in the deep portion. The spores are relatively susceptible to heat, being destroyed by a temperature of 80° C. for one hour. Aside from its occurrence in meat, nothing is known of the life history of the bacillus.

Toxin.

The disease is caused by the toxin which has already been produced in the meat and not by the activity of the organism after it has reached the alimentary tract (v. Ermengem). If an extract of the meat is made with water and the bacteria removed from the latter by filtration, the fluid shows characteristic toxicity for animals. This experiment may be used for determining the presence of botulism toxin in suspected meat. The guinea-pig is the most susceptible animal.

1. Other pathogenic organisms, especially *B. enteritidis* and *B. coli communis*, and recently the paratyphoid bacillus, have been found in poisonous meats. The term botulism formerly was applied to various forms of meat poisoning.

According to v. Ermengem, the bacillus does not proliferate in the body, nor does it produce toxin vigorously at body temperature; hence, he considers it to be a strict saprophyte—a pathogenic saprophyte.

The toxin is taken up by the circulation from the alimentary tract and is not destroyed by the gastric and pancreatic juices, differing in this respect from the toxins of diphtheria and tetanus. It is prepared artificially by growing the organism anaërobically in suitable bouillon and subsequently sterilizing the fluid by filtration. Like the other soluble bacterial toxins, it is susceptible to the action of air and light, and is destroyed by a temperature of from 60 to 70° C.

Pathogenesis. That the toxin has a special affinity for the nervous tissues is evident from the symptoms of the disease; histologically, the ganglionic cells show degeneration in fatal cases. Further evidence of a strong affinity between the toxin and nervous tissue lies in the ability of the latter to neutralize the toxin in the test-glass. The toxin, however, appears not to be so selective in its action on the nervous tissue as the toxin of tetanus, for in botulism degenerations of the glandular organs, and of the vascular endothelium with consequent hemorrhages are characteristic anatomic findings. Man appears to be very susceptible to the intoxication, whereas dogs, rats, and cats are relatively immune. The toxin is pathogenic by subcutaneous or intravascular injection.

Acording to v. Ermengem, the bacilli when inoculated subcutaneously do not proliferate, but are taken up by the phagocytes immediately or after they have been carried to other organs. Animals

which have recovered from infection or which have been immunized acquire rather strong immunity to subsequent inoculations, the immunity being antitoxic.

Prophylaxis and Antitoxin.

The prophylactic measures consist in the avoidance of poorly preserved and improperly cooked meats, especially sausages. Botulism would seem to be very rare in this country where raw meats are not used extensively.

The antitoxin (Kempner) has proved of some value in animal experiments, but its commercial preparation has not been warranted on account of the rarity of the disease.

IV. BACILLUS PYOCYANEUS.

Pathogenic Properties.

For a long time it was thought that the "bacillus of blue pus" was of no importance as an infectious agent for man, although its pathogenicity for animals had been recognized experimentally. It is found with some frequency in the blood and organs of man at autopsy, when death has resulted from some other infection or chronic disease, and in such instances it is supposed that a so-called "agonal invasion" by the organism has occurred. During recent years, however, several cases of primary pyocyaneus septicemia have been observed, the bacillus having been obtained from the blood in pure cultures during life or from the blood and organs shortly after death. It has been found as the sole organism in meningitis and vegetative endocarditis. Some of the cases indicate, however, that a previous lowering of resistance, as that caused by tuberculosis and syphilis, is important for general invasion by the bacillus. It has been found several times in suppurative processes in the middle ear, and would seem to be either the cause

or a strong adjuvant in some cases of severe enteritis, especially in childern. In systemic infections, the symptoms are typhoidal in character, with high temperature, diarrhea and a tendency to the formation of hemorrhages in the skin and internal organs.

Its Manifold Activities.

The *Bacillus pyocyaneus* is widely distributed and that it causes so few infections is probably due to its low pathogenic power. It is an organism of manifold activities. It produces a substance, pyocyanin, which, when exposed to the air, assumes a bluish tint, and on which the color of the pus depends; pyocyanin is soluble in chloroform, from which it may be precipitated in crystalline form. Under proper conditions the organism also forms

Ferments.

a fluorescent pigment. It produces a strong peptonizing ferment, coagulates milk, and in old cultures an autolytic ferment is found which digests many of the bacilli. As stated in a previous chapter, Emmerich and Löwe have identified a bacteriolytic ferment, pyocyanase, which dissolves the anthrax bacillus and other organisms. The ferment nature of this substance is in some doubt, inasmuch as it resists the boiling temperature. Dietrich thinks its action is due to the production of osmotic changes. Old cultures contain a hemolytic agent (pyocyanolysin) of an alkaline nature, which resists boiling and is not a true toxin, since

Toxin and Endotoxin.

immunization with it does not yield an antitoxin (Jordan). In addition to the products mentioned, the organism secretes a true soluble toxin for which it is possible to obtain an antitoxin, and possesses, furthermore, an endotoxin for which an antitoxin can not be obtained.

The soluble toxin of *Bacillus pyocyaneus* is not

produced in large amounts. It differs from the other soluble toxins in its resistance to heat, withstanding a temperature of 100° C. for five minutes. It produces the symptoms which are characteristic of infection with the living organism, the principal anatomic changes being parenchymatous degenerations and ecchymoses, the latter supposedly being due to degenerative changes in the endothelium of the vessels.

Antitoxic and Bactericidal Serums.

By immunizing with young cultures grown on an agar surface a serum which is purely bactericidal is obtained. On the other hand, if an older toxin-containing bouillon culture be used, the serum is both bactericidal and antitoxic. The serum which is purely bactericidal has no power of neutralizing the toxin. The toxin solution contains not only the true toxin, but also quantities of endotoxin which were liberated as the dead bacilli were dissolved. Inasmuch as the antitoxin neutralizes only the true toxin, leaving the endotoxin unbound, the toxicity of the filtrate can not be destroyed entirely by antitoxin, a condition which is brought out clearly when the attempt is made to neutralize a multiple of the simple fatal dose by the corresponding amount of antitoxin. In such multiples a fatal amount of endotoxin is present. Although a strong antitoxin may be obtained, it would appear to be of little practical importance because of the rarity of infections by the bacillus.

Agglutination.

Infection in man has caused the formation of agglutinin in several instances, but it has been absent in others. An agglutinating serum is readily produced by artificial immunization.

V. OTHER SOLUBLE BACTERIAL TOXINS.

Soluble toxins, of perhaps secondary importance, which are produced by the staphylococcus and streptococcus, will be considered in the sections dealing with these organisms. It seems probable that they do not represent the essential toxic agents of the cocci, but rather that the toxicity of the latter depends chiefly on the action of endotoxins.

B. INTOXICATION BY SOLUBLE PLANT TOXINS.

I. HAY FEVER.

Dunbar separated from the pollen of various grains a toxin which is able to precipitate typical attacks of hay fever in those who are susceptible, having first demonstrated that the crude pollens cause the disease. The pollen from the following are said to contain the toxin: Rye, barley, wheat, maize (corn), dog's tail, couch-grass, millet, rice and some others. The so-called autumn-catarrh which is common in America may be due to a slightly different toxin coming from the golden-rod, rag-weed, and perhaps other autumnal flowering grains.

The Toxin. The toxin usually is associated with certain starch-like granules which are contained in the pollen, but it occurs also in pollens which do not contain these granules. It may be extracted with water or salt solution, is precipitated by alcohol, resists the boiling temperature, and is of an albuminous nature.

Pathogenesis. When the crude pollen reaches the conjunctiva, nasal or bronchial mucous membranes, the toxin is dissolved out by the secretions and absorbed by the lymphatics. When applied to the conjunctiva

it causes swelling, redness and lachrymation. It is carried by the tears to the nose and here causes excessive secretion, swelling of the mucous membrane and sneezing. It may become distributed systemically as a result of absorption from the free surfaces and cause the asthmatic attacks and general symptoms which are seen in the intoxication. When injected subcutaneously into the arm both the asthmatic attacks and coryza-like symptoms were produced.

Antitoxic Serum (Pollantin.)

Dunbar's antitoxic serum (pollantin) is obtained by immunizing horses with the toxin. It seems to be of undoubted value in a certain percentage of cases, but fails unaccountably at times. It is, perhaps, most effective when used in the prodromal stage, the attacks being thereby prevented. Its failure in certain instances may be due in part to the inefficacy of the antitoxin against the toxins of certain pollens. Again, in certain individuals the affinity of the toxin for the tissues may be unusually great so that a more vigorous use of the remedy is demanded.

Lübbart and Prausnitz published statistics of 285 cases, of which 65 were autumnal. In ordinary hay-fever the serum gave positive results in 57 per cent., partially positive in 32 per cent. and negative results in 11 per cent. of the cases. In autumnal catarrh, 70 per cent. were positive, 19 per cent. partially positive, and 11 per cent. negative.

The small bottles of antitoxin are accompanied by a pipette with which from one to several drops may be instilled into the eye or the nose.

The serum does not cure permanently and one who is susceptible should carry a vial for immediate use during the hay-fever season.

II. OTHER PLANT TOXINS.

Ricin, from the seeds of *Ricinus communis;* abrin, from *Abrus precatorius;* crotin from the seeds of *Croton tiglium;* and robin, from the leaves and bark of the locust tree (*Robinia pseudoacacia*) are chiefly of experimental interest. They are similar in their action, are very toxic to animals, producing both local and general changes with fatal termination when given in sufficient doses; they have pronounced agglutinating action on the erythrocytes of most animals, and in some instances are slightly hemolytic. By guarded immunization antitoxins may be obtained for them.

Kobert gave the name of *phallin* to a toxic substance which may be extracted from poisonous mushrooms, particularly the "Deadly Amanite" (*Amanita phalloides*). In some countries many deaths are caused by eating this variety: Russia, Germany, Italy, France, Japan (Ford). Phallin is very toxic for animals and is strongly hemolytic for many bloods. By immunization Ford has recently obtained an antitoxin which neutralizes the hemolytic action of the poison, and which in a dose of 0.5 c.c. protects rabbits against five fatal doses of the toxin. The toxin is an aqueous extract of the dried plants.

C. INTOXICATION BY SOLUBLE ANIMAL TOXINS.

I. POISONING BY SNAKE BITES.

The poison apparatus of snakes consists of a secretory gland on each side which communicates with a tubular fang by means of a duct. In the

Toxic Constituents.

passive state the fangs are directed backward on the roof of the mouth, but when the animal strikes their points are made to project forward and the poison is forced through the canals by muscular compression of the sac. The venom is a glandular secretion.

The venoms of different snakes vary a great deal in their toxic properties. The most important constituents are those which attack the nervous system (neurotoxin), the blood corpuscles (hemolysins and hemagglutinins) and the endothelium of the blood vessels, causing hemorrhages (hemorrhagin, an endotheliotoxin). The three are independent.

The neurotoxin causes death by paralysis of the cardiac and respiratory centers. The hemolysin appears to be of less importance as a cause of death.

Variations in Toxic Properties and Cytotoxins.

The venoms of the cobra, water-moccasin, daboia and some poisonous sea-snakes are essentially neurotoxic, although they have strong dissolving powers for the erythrocytes of some animals. In studying the hemolytic powers of the venoms of cobra, copperhead and rattlesnake, Flexner and Noguchi found cobra venom to be the most hemolytic and that of the rattlesnake the least. They attribute the toxicity of rattlesnake poison chiefly to the action of hemorrhagin. The same authors studied the action of different venoms on the cells of various animals and by absorption experiments found independent cytotoxins for the testis, liver, kidney and blood. Not only was there a distinct cytotoxin for each organ of an animal, but also for the same organ of different animals, results which speak for a remarkable complexity of

venom. Certain venoms contain a leucocytic toxin.

Ferments. That venoms contain proteolytic ferments is shown by their ability to digest gelatin and fibrin. This power may be related to the softening of the muscles which has been noted clinically in cases of poisoning. The rapid decomposition of the body which follows death by snake-poisoning is associated with a decrease in the bactericidal power of the blood, which, according to Flexner and Noguchi depends on fixation of the complement by the venom.

Amboceptors and Complement. The hemolysin and neurotoxin, and perhaps other cytolysins of venom, consist of amboceptors which in themselves are non-toxic; they become toxic only through the aid of complements which are present in the body of the poisoned animal. In this instance, complement which usually is a source of protection becomes a source of danger to the animal possessing it. Not only does ordinary serum-complement serve for activation, but Kyes discovered that cells (erythrocytes) may contain another kind of complement, an "endocomplement," which activates the amboceptors after the latter have combined with the cells. Flexner and Noguchi found that this also was the case with the neurotoxic amboceptors.

The ability of lecithin to activate the hemolytic amboceptors of cobra venom and the preparation of cobra-lecithid (Kyes) were described in Part I, Chapter XII, pages 158-160. In the preparation of cobra-lecithid the neurotoxin is separated from the hemolysin, the former remaining in solution, whereas the latter settles as a precipitate in combination with the lecithin. Immunization with

the neurotoxin isolated in this way causes the formation of a specific antineurotoxin (Elliot). The neurotoxin may also be abstracted from the venom by treating the latter with the nervous tissue of a susceptible animal (Flexner and Noguchi).

The hemolysin is distinct from the hemagglutinin and the latter may be eliminated by heating the venom to from 75° to 80° C. In the action of venom on erythrocytes agglutination precedes hemolysis.

Toxoids and Antivenins.

The toxins may be converted into toxoids by heat or treatment with chemicals. Immunization with toxoids causes the formation of antitoxins. Radium is said to destroy the toxicity of venom (Physalix).

The antivenin of Calmette is obtained by immunizing horses with a mixture of venoms (80 per cent. cobra, 20 per cent. viperine venom) which are attenuated before injection. Six months are required to produce a strong serum. The claim of Calmette that his serum is effective against all snake-venoms is erroneous. It neutralizes those venoms the toxicity of which depends largely on neurotoxins and hemolysins, but has little influence on rattlesnake poison, the essential toxin of which is hemorrhagin. Antivenin for the rattlesnake and water-moccasin may be prepared by immunization with the corresponding venoms which have been attenuated by weak acids. Noguchi has produced serum of such strength that it promises to be of practical value in the treatment of rattlesnake bites.

As indicated previously, the action of venom is preceded by no appreciable incubation period; hence, an antitoxin to be effective must be admin-

istered not later than a few hours after the bite has occurred. Noguchi found in relation to antivenin for the rattlesnake that the antitoxin necessary to save was quadrupled three hours after intravenous injection of two fatal doses of venom. Fortunately the venom is less toxic when introduced subcutaneously.

II. OTHER ZOOTOXINS.

Phrynolysin, which is present in the blood and skin of certain toads, has been studied especially by Pröscher. It is a thermolabile, hemolytic toxin for which an antitoxin can be obtained by immunization.

Arachnolysin, obtained from the bodies of certain spiders, is a hemolytic toxin, which by immunization yields a specific antitoxin.

A poison, with properties resembling those of snake venom, may be obtained from the caudal segment of the scorpion. Antitoxin is produced by immunization.

Ichthyotoxin, a name given to the toxic properties of eel serum, is composed of a neurotoxic and a hemotoxic constituent.

From the poisonous glands of certain fish (*Trachinus draco*) a highly toxic, thermolabile substance is obtainable, for which an antitoxin can be prepared by the immunization of rabbits.

GROUP II.

Acute infectious diseases caused by bacteria which do not secrete strong soluble toxins in culture media, but which contain endotoxins (toxic protoplasm). Infection or immunization causes immunity of considerable or prolonged duration. In active immunity the serums agglutinate the corresponding organisms and are protective for other animals,* but have little or no curative power. The formation of antitoxins is not definitely established. In most instances vaccination has been accomplished. Clinically there is leucocytosis in some instances and hypoleucocytosis in others (typhoid and Malta fever).

A. The serum in acquired immunity is bactericidal.

I. TYPHOID FEVER.

Eberth first saw *Bacillus typhosus* in microscopic preparations of the mesenteric lymph glands and spleen of a typhoid corpse, in 1880. Koch also observed it at about the same time, and stained it in the intestinal wall, spleen, liver and kidney. It was obtained in pure culture by Gaffky in 1884.

The organism is rod-shaped, 0.5 to 0.8 by from 1 to 3 microns in dimensions, with nothing characteristic in its morphology. It possesses from ten to twelve flagellæ situated at the end and on the sides and is actively motile under suitable conditions. It forms no spores and is readily cultivated on many media.

The bacillus is one of the rather numerous "in-

* This has not been established in regard to Malta fever.

testinal group" of organisms, certain members of which are so similar that they can be differentiated only by means of special culture manipulations, animal experiments, or the agglutinating and bactericidal action of specific immune serums.[1]

Distribution of the Bacillus.

The organism has been cultivated from earth and infected water, and from the feces, urine, blood, rose-spots and the various organs of typhoid patients. In many instances in which an epidemic has certainly been caused by an infected water supply attempts to cultivate the bacillus from the water have failed. The organisms may not have been included in the samples which were analyzed, or, what is equally probable in certain instances, they have died out in the water by the time the disease was so widespread as to be considered epidemic. Its occurrence in nature depends on the distribution of the infected excretions of the diseased.

Viability and Resistance.

The viability and virulence of the bacillus in water, earth, etc., vary with the nature of its surroundings. It has been found to live for periods of from 2 to 4 weeks to 2 or 3 months in water, from 3 to 4 months in milk, from 3 to 5 months in surface water, and from 11 to 16 months in sterilized earth; 100 days in ice, from 12 to 30 days in oysters, from 50 to 80 days when dried on clothing, for 3 months in typhoid feces, for 96 days in the dead body of an experiment animal. When in water or moist earth which contain many saprophytes its life is shortened. It survives drying for many months, al-

1. Of this group the bacillus of dysentery, paratyphoid bacillus, *Bacillus enteritidis* of Gärtner, colon bacillus and *Bacillus alcaligenes*, in addition to the typhoid bacillus, are the most important because of their similar morphologic and cultural properties and the pathogenicity of certain of them.

though direct sunlight kills in the course of a few hours.

Endotoxin.

The typhoid bacillus secretes no soluble toxin, but contains an endotoxin which may be obtained in solution by the autolytic digestion of cultures, by extracting ground-up bacilli or by squeezing out the plasma under high pressure. Up to the present time, immunization with none of these preparations has resulted in the production of an antitoxic serum of accepted value.

Typhoid Epidemics.

Typhoid fever may become epidemic either through a contaminated water supply or by contact infection. When due to infected water there is something characteristic about the explosive-like suddenness with which dozens or even hundreds are stricken within a short period. The water of streams, small lakes or reservoirs may become infected from an ill-constructed out-house, or from discharges which have been thrown on the ground in their vicinity. Typhoid stools thrown on the ground adjacent to wells have caused miniature epidemics. Fruit, vegetables and milk cans may be infected by washing them with contaminated water, and it is supposed that the disease may be acquired from oysters which have lain in water contaminated with sewage.

By whatever means an epidemic is set in motion, primarily, it is usually aggravated and prolonged by the occurrence of contact infections (indirect contact). The hands of the nurse, physician, or others who come in contact with the patient become contaminated from the stools, urine, soiled linen or skin of the patient, and the organisms subsequently are transferred to food, drinking water, or in other accidental ways reach the mouth.

Each new case is a fresh focus from which infection may be carried to others, and the chances of milk and food infection become greater as the cases multiply. When the discharges are not disinfected or are improperly disposed of, soil or house infection may occur and the possibility of transmission by germ-laden dust becomes of importance. Dust infection from dried urine or feces and drop infection from urine, water, or the sputum of the patient are theoretically possible, but would seem to be of minor significance. That flies may carry the organisms from open vaults or cesspools and deposit them on food or in drinking water has been appreciated in relation to epidemics in military camps. Typhoid bacilli have been cultivated from flies which were taken from the vicinity of infected material.

The Infection Atrium.

The micro-organisms gain access to the body through the lymphoid tissue of the intestinal tract (Peyer's patches and the solitary follicles). The occurrence of primary infection of the lungs through inhalation of infected dust is possible, but has not been definitely proved. In this instance typhoid bacillemia might occur either with or without intestinal infection. In the latter case it would seem essential that some local lesion exist in the lungs or elsewhere from which organisms could constantly be supplied to the blood. Neufeld doubts the ability of the typhoid bacillus to proliferate in the blood, because of the strong bactericidal power of the latter, and considers that infection takes place through the intestines even in cases of "typhoid without intestinal lesions."

Incubation Period

The incubation period is subject to considerable variations. In a series of cases in which the date

of exposure was known, 62 per cent. showed symptoms in from 20 to 25 days, 2 per cent. in from 14 to 20 days, and 2 per cent. later than 30 days.

Localization of the Bacilli.

Following the development of intestinal lesions, the bacilli reach the circulation by way of the lymphatics, and through the action of the bactericidal constituents of the blood (amboceptor-complement complex and possibly leucocytes) they are killed and dissolved in large quantities. It is now generally believed that only through the disintegration of the bacterial cells are their toxic constituents thrown into solution in the body, a condition which is necessary in order that the tissues be injured. Infection of the blood stream with living organisms, in the early stages of the disease and preceding relapses, occurs in a large percentage of the cases.

Diagnosis by Blood Cultures.

It is possible to establish the diagnosis of typhoid fever by cultivating the bacilli from the blood, even before the serum has developed sufficient agglutinating power to give a positive Widal reaction. A small flask of bouillon is inoculated with from 1 to 5 c.c. of blood, drawn from the median vein of the arm, and after twenty-four hours of incubation a small portion of it is plated out. Colonies which devlop on the plates may be identified by the usual bacteriologic methods, or the agglutination test may be performed with a known antityphoid serum. After from the tenth to the fourteenth day the organisms can rarely be cultivated from the blood; the bactericidal substances of the blood may have so increased by this time that circulating bacilli are killed rapidly.

In from one-fourth to one-third of the cases, in the third week, or during convalescence, the bacilli

appear in large numbers in the urine, in which they may persist for many weeks. According to Kanjajeff, they are discharged into the urine from metastatic foci in the kidneys.

Many of the symptoms, complications and sequelæ of typhoid fever, as the rose-spots, enlarged spleen, bone lesions, and in some instances nervous lesions and pneumonia, depend on the distribution of the bacilli. This is in contrast to the conditions in diphtheria and tetanus, in which the distribution of the bacilli is of little significance for the involvement of particular organs. The anatomic changes and clinical symptoms suggest that the lymphoid tissue and central nervous system have a special affinity for the toxic constituents of the typhoid bacilli.

Endothelial Hyperplasia.

The greatest changes take place in the organs (lymphoid) which contain the bacilli most constantly and in the greatest numbers. It is here that the toxic substance may be present in greatest concentration, as a consequence of the continual solution of the organisms. Mallory describes an enormous hyperplasia of the endothelial cells, especially those of the lymphatic structures. The cells are phagocytic, and especially in the lymphoid tissue of the intestines and in the mesenteric lymph glands, englobe and destroy the lymphoid cells on a large scale. It seems probable that the endothelial proliferation which has been described is due to the rather mild but prolonged action of the dissolved toxic constituents of the typhoid bacillus; the condition is that of an inflammatory hyperplasia. It has been suggested that the hypoleucocytosis of typhoid fever is due to the destruction of the lymphocytes in the lymphoid organs by the endothelial phagocytes.

The granular and fatty degenerations of the parenchymatous organs do not differ from those seen in many acute infections.

Mixed Infections.

The conditions in the intestinal tract would seem to favor mixed infections, especially by the colon bacillus and streptococcus, and the primary infection probably decreases the resistance to secondary invasion. The rôle of the colon bacillus in typhoid fever is perhaps not definitely established, although it has been found in the circulation, in abscesses, and in the urine in cases of cystitis accompanying the disease. The typhoid and colon bacilli grow well together. A mixed general infection with the streptococcus causes a grave septic condition characterized by an irregular temperature curve. This condition may be discovered by blood cultures. It is thought that the streptococcus does not increase the toxicity of the typhoid bacillus, the result being rather a summation of the intoxication of the two infections. Post-typhoidal suppurations are often due to the streptococcus and in many of the metastatic complications (parotitis, pleurisy, peritonitis, meningitis, otitis media) streptococci and staphylococci have been found. Pneumococcus pneumonia not infrequently complicates typhoid fever. A combined infection of typhoid and malaria is said to occur in the tropics; the complication is grave. Typhoid and diphtheria may occur together, and typhoid may be superimposed on acute tuberculosis.

Immunity and Susceptibility.

The period of greatest susceptibility to typhoid is found from the fifteenth to the twenty-fifth years. The resistance of infants and children is not satisfactorily explained. A certain amount of resistance inherited from the mother may persist

for some years after birth. It is known that antibodies may pass from the mother to the fetus through the placenta. In very early life the tissues may respond more energetically to incipient infection by the rapid formation of typhoid antibodies, or the phagocytic cells may be more active. The conditions which render older people less susceptible are no better understood. A loss of suitable receptors may have occurred so that the toxic constituents of the bacilli find no anchorage in the body, or the affinity between the receptors and the toxic constituents may have become less. The individual during the course of years may have been gradually immunized by the entrance of nonpathogenic quantities of the bacilli into the circulation. That resistance to typhoid infection is decreased by low nutrition and overwork is a long-known fact.

Natural and Acquired Immunity.

A large amount of protection is afforded by the hydrochloric acid of the gastric juice, and it is reasonable to believe that suppression or an insufficient amount of hydrochloric acid may favor the passage of living bacilli to the intestines. Normal human serum is rather strongly bactericidal for the typhoid bacillus, and the leucocytes ingest and destroy it. Metchnikoff ascribes natural immunity to the action of the microphages.

Duration of Acquired Immunity.

The immunity which follows an attack of typhoid fever is generally of long duration, but second attacks occur with some frequency. It has been noted that limited communities which have experienced an epidemic may remain relatively free from the disease over a period of some years, although neighboring districts are attacked. All the susceptible persons having had the disease, a

state of temporary regional immunity is created. Acquired immunity is characterized by an increase of the bactericidal amboceptors, agglutinins and typhoid precipitins in the serum. It is commonly believed that recovery is due to the increase of the bactericidal power of the body fluids, which becomes most marked during the later period of the disease or during convalescence. It seems certain, however, that the new resistance persists beyond the time when the bactericidal power of the serum has returned to normal, which may take place in from one to several years. The bactericidal power sinks rapidly during and following convalescence. However, the general principle is well established that, although the antibodies may have disappeared entirely, they are reformed more readily as a consequence of an old infection (Neisser and Shiga). The tissue cells have, so to say, been trained, and are stimulated by a few micro-organisms to produce such a quantity of bactericidal amboceptors that the incipient infection is overcome. It is, of course, understood that the amboceptors require the aid of complement in killing the micro-organisms. A second attack of typhoid fever usually is mild.

Leucocytes.

Metchnikoff does not deny that the amboceptors (fixators) play an important part in acquired immunity, but claims that the new resistance depends chiefly on an increase in the phagocytic power of the microphages (polymorphonuclear leucocytes). This is not clear from the clinical standpoint because of the hypoleucocytosis which is somewhat characteristic of typhoid—a hypoleucocytosis caused chiefly by a disappearance of the microphages. It has been suggested that our con-

clusions as to hypoleucocytosis are based on examination of the peripheral blood, whereas the mesenteric vessels may show hyperleucocytosis. Mallory, however, found a striking absence of microphages even in the intestinal vessels. Concerning a theory that the hyperplasia of the lymphoid organs serves as a substitute for the hyperleucocytosis, we may recall the findings of Mallory that this hyperplasia is chiefly one of endothelial cells. The importance of these endothelial cells for the destruction of typhoid bacilli needs further investigation.

Prophylaxis. Prophylaxis should begin with the thorough disinfection of the stools and urine of typhoid patients, and this should be continued until they no longer contain typhoid bacilli. It is not good hygiene to discharge a patient until bacteriologic examination of stools and urine show them to be free from the organisms. It would be difficult to carry out this rigid precaution under all conditions, but at all events the stools and urine may be disinfected for a reasonable period, say throughout convalescence. There is no sufficient reason for the neglect of the bacteriologic examination in hospital practice. There is a growing sentiment that typhoid patients in hospitals should be isolated in wards or rooms in which there is a fixed routine for the disposal of infectious materials—urine, stools and sputum. Soiled linen, the bath water of typhoid patients, the remnants of food and drink, and the eating utensils should be disinfected before removal from the room. Nurses or attendants should not eat or drink in typhoid rooms.

Hexamethylenamine.

The value of hexamethylenamine in causing the disappearance of bacilli from the urine is now well known, and the advisability of using the drug as a routine measure for public safety is worthy of consideration. The room should be kept free from flies and eventually it should be disinfected, preferably by formalin. During an epidemic, in case the water supply of a community is susceptible to contamination ,all water used for drinking, washing of vegetables and eating utensils, should be boiled, and that used for general cleaning may be otherwise disinfected. The possibility of dust infection of a house should not be disregarded.

Serum Therapy and Vaccination.

There are two methods of specific prophylaxis against typhoid: 1, the injection of antityphoid immune serum; 2, preventive inoculation with killed cultures of the bacilli. Antityphoid serum confers a fairly strong and immediate immunity which, however, is of short duration, because of the rapid elimination of the serum. Its use as a general preventive, therefore, is not advocated.

Wright's Method and Results.

Wright has been influential in showing the utility of protective inoculations against typhoid. His first experimental work was published in 1896. Since that time the inoculations have been carried on extensively in British regiments in India and South Africa. The occurrence of typhoid among the inoculated was one-half that among the uninoculated, and the inoculations reduced the mortality of the disease by one-half. The protection, so far as known, lasts for two or more years, although in some instances infection has occurred in from three to six months after vaccination.

The Vaccine.

The methods of preparation of the vaccine are elaborate in order to insure sterility and standard-

ization. Cultures of the bacillus are grown in bouillon for from twenty-four to forty-eight hours, and then sterilized at 60 C. The contents of several flasks are mixed in order to obtain a uniform distribution of organisms, and standardization is then accomplished by a convenient method of estimating the number of bacilli in a cubic centimeter of the vaccine. The purity of the vaccine is insured by bacteriologic tests, and for preservation carbolic acid or lysol is added.

Effects. Wright has abandoned his original method of giving a single injection and now recommends two moderate doses, which are given from eight to fourteen days apart. The first dose includes a quantity of vaccine which contains from 750,000,000 to 1,000,000,000 of bacilli, the second 1,500,000,000 to 2,000,000,000. Wright finds that "the inoculation of these quanta induces an ample elaboration of antitropic substances (antibodies) without producing any severe constitutional reaction." The inoculations increase the bactericidal and agglutinating powers of the serum and it is concluded that an increased resistance to typhoid intoxication is established because the second injection causes milder symptoms than the first. The phagocytic power of the leucocytes is raised, because of an increase in the "opsonic antitropins" (Part I, Chapter XIV). The curve of the antibodies is like that usually obtained by active immunization with bacteria, toxins or other substances. Immediately following the inoculation there is a decrease even of normal antibodies. This "negative phase" lasts for from one to several days and corresponds to a period of increased susceptibility. It is quickly followed by a positive phase

in which the antibodies and, correspondingly, the resistance increase rapidly. When very small doses are administered the positive phase may be recognized after twenty-four hours (Wright). Large doses cause a prolonged negative phase and are to be avoided.

Local Reactions.

Following injection, "the local symptoms first make themselves felt after an interval of two or three hours. The effects then seen are the development of a red blush and more or less serous exudation at the site of inoculation, followed by some lymphangitis along the lymphatics which lead, according as the vaccine has been inoculated above or below the middle line of the trunk, in the direction of the glands of the axillæ or of the groin. . . . Even severe inflammation has never led on to suppuration." The exudate is somewhat hemorrhagic, and the pain moderate to severe, but not of long duration. With the technic as recommended at present, "the constitutional symptoms are limited to some headache and to two or three hours of real malaise. . . . The next day his temperature comes down to normal, and he feels comparatively well except in respect to pain at the seat of inoculation."

General Reaction.

Conditions for Vaccination.

The adoption of antityphoid inoculation or vaccination under certain conditions appears to be warranted. Typhoid never has been a world pest; hence, the occasion for universal vaccination does not exist, but in the presence of epidemics so frequently seen in American cities it will be impossible to avoid the consideration of vaccination as a means of protecting the uninfected. The question is a pertinent one also for those cities in which typhoid is so extensive as to be called endemic.

Mixed Active and Passive Immunization.

It has been suggested that the "negative phase" described above is a source of danger in the presence of an epidemic. The phase is so short, however, that the danger is minimal and it seems probable that the practice of mixed active and passive immunization would eliminate it entirely. This is accomplished by the combined injection of antityphoid serum and vaccine. The serum assures a positive phase from the start, and before this has subsided, that induced by the vaccine is established. When specific serum is mixed with the vaccine the local reaction is said to be less severe.

The products of autodigestion of typhoid cultures have been suggested as suitable vaccine (Neisser and Shiga). The local reaction is said to be mild, and the body reacts by the formation of bactericidal amboceptors and agglutinins.

Serum Therapy.

Bactericidal serums obtained by the immunization of horses with typhoid bacilli have not shown distinct curative properties. Chantemesse immunizes horses with a typhoid "toxin" which is prepared by growing the organism in a liquid culture which contains an emulsion of splenic tissue. One cubic centimeter of this toxin will kill a guinea-pig, a dose which in comparison with other bacterial toxins is very weak. Chantemesse has used his antitoxic serum in the treatment of more than 500 cases, reporting a mortality of about 6 per cent., whereas that among untreated patients was from 10 per cent. to 12 per cent. Although these figures indicate some value for the serum it has had little trial outside of France.

McFadyan and Rowland immunize horses with extracts of typhoid bacilli, which have been ground

up while they were kept in a brittle state by the temperature of liquid air. Although antitoxic and bactericidal properties are claimed for the serum, there is no conclusive evidence that it differs from a bactericidal serum prepared in the ordinary way.

Preparation of Jez.

Jez produces a high degree of immunity in rabbits by artificial immunization with the typhoid bacillus, then prepares an extract from the spleen, bone marrow, brain, etc., of the immunized animals. The extract is administered by mouth. Jez justifies this method, from the fact that the lymphoid organs have been shown to form typhoid antibodies (Wasserman). From the clinic of Eichorst and some others favorable reports concerning the remedy have been published. It has had no extensive use. The preparation is made by the Serum Institute of Berne (Switzerland) and is expensive.

The suggestion made by Fraenkel, that typhoid patients be treated by subcutaneous injections of small quantities of killed typhoid bacilli in order to hasten the formation of antibodies has been kept alive through the "typhoin" of Petruschky, but is yet without much practical trial. Of a similar nature is the suggestion of Richardson, that the filtrates of typhoid cultures be injected.

Agglutination

The principles and technic of the agglutination test were described in Part I. The serum commonly becomes agglutinating on from the seventh to the tenth day, rarely as early as the second or third, and as late as from the twentieth to the fortieth day. The power is highest during convalescence, when it may agglutinate in dilutions as high as 1/5,000 or higher, and from that time sinks gradually. An agglutinating

power of 1/160 has often been found at eight months, and of 1/50 after from seven and one-half to eleven years; but the latter duration is not the rule. In performing the test a serum dilution of not less than 1 to 40, or 1 to 50 should be observed as previously set forth.

The following sources of error are to be borne in mind: Typhoid fever occasionally runs its course without the formation of agglutinins; the reaction may mysteriously be absent one day to recur a few days later, a condition which indicates the importance of repeated tests; rather high agglutinating power for the typhoid bacillus occasionally develops in other infections, as pneumonia, meningitis, icterus, Weil's disease, etc.; the possibility of group agglutination, for the positive elimination of which control tests with related organisms may be demanded.

In case negative results are obtained in a suspicious case, the reactions should be tried with the paratyphoid bacilli.

The test of the bactericidal powers of the serum has been recommended as a substitute for the agglutination reaction, but the technic is so much more complicated that the method will probably not come into general use.

For diagnosis previous to the formation of agglutinins, blood cultures should be made as described in a preceding paragraph.

II. PARATYPHOID FEVER.

Paratyphoid and "Paracolon" Bacilli.

In 1900 Schottmüller cultivated from the blood of five "typhoid" patients organisms which differ from the typhoid bacillus in that they attack dextrose with gas formation and are not agglutinated conspicuously by antityphoid serum. Since that

time many similar cases have been reported and two types of the paratyphoid bacillus have been recognized (Schottmüller). Group B causes first an acid reaction in milk which changes to a permanently alkaline reaction in about ten days, whereas Group A causes permanent acidity (Kayser). They resemble the typhoid bacillus morphologically, but culturally are more closely related to to *Bacillus enteritidis.* Organisms which have previously been described as "paracolon" bacilli (Widal, Gwyn) do not differ from those which are now called paratyphoid bacilli, and the infections caused by them resembled the recorded cases of paratyphoid fever. The term "paracolon" should no longer be applied to them.

Paratyphoid fever occurs sporadically or in epidemic form, and bears a close resemblance to mild typhoid-like epidemics which have been noted from time to time, and which, presumably, are caused by eating poisonous meats. One such epidemic of 600 cases was caused in Switzerland in 1878 by the meat of a sick calf; the mortality was 1 per cent. A still older epidemic (1839) is cited, likewise caused by meat. In both instances the infection eventually was carried from person to person by contact. A recent outbreak in Kiel, proved to be paratyphoid, is assumed by Fischer to have been caused by infected meat, on account of the peculiar distribution of the cases among the patrons of a particular market. Kurth also attributed a small epidemic to either uncooked meat or milk. Fischer mentions 50 cases in East Holstein which probably were infected by the milk of two cows. Shortly after the epidemic began the cows died and paratyphoid bacilli were cultivated from the muscles,

Epidemiology.

spleen, liver and intestines. De Feyfer cites an instance in which the disease apparently was transmitted through the water of a stream in which the clothing of the first patients had been washed. In another instance, a regimental infection was traced to the discharges of a single soldier, the water supply having become contaminated through a defective water closet.

Characteristics of the Disease.

Paratyphoid, like typhoid fever, is accompanied by an enlarged spleen and many rose spots. Although severe symptoms may be present for a time, the course of the disease usually is mild and the mortality is low. The incubation period approximates that of typhoid. In the few cases which have come to autopsy the intestinal lesions have varied from a mild ileocolitis with an intact mucous surface to a condition of superficial ulceration. The involvement of Peyer's patches and the solitary follicles which is so characteristic of typhoid is absent, although these structures may be moderately swollen. The mesenteric lymph glands are not markedly involved and there is little proliferation of the lymphoid or endothelial cells (Wells and Scott). The disease has no specific anatomic lesion.

Excretion, Resistance and Distribution.

The organisms are found in the blood and various organs, in the rose spots, urine and feces of the patients. Practically nothing is known of the occurrence of the bacilli outside the body. Because of their presence in the stools and urine of the patients, the methods of dissemination and infection doubtless are similar to those concerned in typhoid. The bacillus is said to have a marked resistance to heat, withstanding 60° C. for 30 minutes and not all cells being killed during one hour at

this temperature. This may explain the fact that the virus is not always killed by cooking the meat. The organism probably has a wide distribution because of the occurrence of the infection in various parts of the world.

The toxicity of the bacilli depends on the existence of a fixed endotoxin; a soluble toxin is not produced.

The principles of prophylaxis against typhoid also apply to paratyphoid fever, with the addition that in the latter disease the possibility of meat infection must be kept in mind.

The serums of patients and immunized animals acquire bactericidal and agglutinating powers for the organism.

There is no serum therapy for the infection, nor has the occasion arisen to attempt vaccination.

Agglutination and Blood Cultures.

Serum from a paratyphoid patient may agglutinate the homologous bacillus in a dilution of 1/1000 or 1/2000 or more (E. H. Ruediger), whereas the typhoid bacillus is agglutinated only in low dilutions by the same serum. However, bacillus A and bacillus B are not identical in their agglutinable properties; in this respect it is stated that the latter is more closely related to the typhoid bacillus than the former. The agglutination test is said to have a higher diagnostic value than the Gruber-Widal reaction in typhoid, a stronger agglutinating power being developed in the serum of the patient. Nevertheless, the formation of coagglutinins may render the test confusing if proper serum dilution is not practiced. Conclusions should not be attempted until the test has been performed with both strains of the paratyphoid bacillus and with the typhoid

bacillus. As in typhoid, early diagnosis may be best accomplished by bacteriologic examination of the blood.

III. ACUTE EPIDEMIC DYSENTERY.

In addition to amoebic dysentery, we have become familiar with an acute dysenteric infection which appears epidemically in both tropical and temperate climates, and prevails especially in the summer months. Such epidemics occur extensively in Japan, where the mortality may be 24 per cent.; in the Philippines, United States, Germany and other European countries. In industrial settlements in Germany the mortality is about 10 per cent. (Kruse). The incubation period may be as short as two or three days. In mild cases the patient may recover in from four to eight days, whereas severe cases last from two to four weeks, and may terminate fatally. Occasionally the infection lasts sufficiently long to be considered chronic.

Two Types of Bacilli.

In 1898, Shiga, basing his conclusions on positive results with the agglutination test and on the constant presence of the organism in the stools of the infected, identified as the cause of the disease, in Japan, a microbe which is known as *Bacillus dysenteriæ* (Shiga). Flexner, in 1900, made similar observations on epidemic dysentery in Manila, and his organisms, or one of them, differing slightly from that of Shiga, is called *Bacillus dysenteriæ* (Flexner), or the Flexner-Harris bacillus, Harris being the name of a patient from whom this typical strain was cultivated. Kruse (1901) found both the Shiga and Flexner types in Germany, needlessly giving the name of "pseudodysentery" bacilli to the latter. In this country similar organ-

isms have been found as the cause of institutional dysentery by Vedder and Duval, of summer diarrheas of infants by Duval and Bassett, and by Wolstein. It is the belief of Vedder and Duval that acute dysentery, the world over, "whether sporadic, institutional or epidemic, is caused by the dysentery bacillus." We must note, however, that the organism is not found in all cases of clinical dysentery, even by skilled bacteriologists. "Clinically, 24 of our 97 cases in which the dysentery bacilli were found did not differ from the cases of ileocolitis in which the dysentery bacilli were not found." (Weaver and others.) It seems certain, nevertheless, that *Bacillus dysenteriæ* is the most important cause of acute dysentery. It rarely occurs in the stools of healthy individuals.

Summer Diarrheas.

The organisms of Shiga and Flexner differ in their actions on sugars (i. e., in their acid-forming powers) and in their agglutinability; the "Flexner" type is the stronger acid-former. An artificially produced immune serum which is specific for one organism has rather higher agglutinating and bactericidal powers for the corresponding type, but low for the other. In this country the "Flexner" bacillus is much more common than that of "Shiga," but here and abroad both types are met, and sometimes in the same individual. Several other organisms have been cultivated from dysenteric patients, but the variations from these two types are slight. All are certainly very closely related.

Characteristics of the Bacilli.

The organism is somewhat thicker than the typhoid bacillus, but probably is non-motile, although Vedder and Duval, in opposition to others (Lentz), claim to have demonstrated flagella. It

often shows a polymorphous appearance in cultures, but forms no spores. It is Gram-negative. It lives for from 12 to 17 days when dried (Pfuhl); direct sunlight kills it in 30 minutes, 1 per cent. carbolic acid in 30 minutes, 5 per cent. carbolic acid plus corrosive sublimate (1/2000) almost instantaneously. It is thought that it may live over winter and cause fresh outbreaks in the spring (Kruse).

Distribution in the Body. The bacillus is found only in the stools of the infected, in the mucous or muco-hemorrhagic portions of which it exists almost in pure culture, few colon bacilli being in the immediate vicinity; it has not been found in the blood or urine. In fatal cases, Shiga found it only in the intestinal ulcers and swollen lymphoid structures and in the mesenteric lymph glands. Flexner mentions its occurrence in the liver. The organism, if it reaches the circulation at all, either does so in small quantities, or is rapidly destroyed by the blood. The infection resembles cholera, but differs from typhoid and paratyphoid in this respect. An observation by Markwald (cited by Lentz) indicates, however, that the bacilli may reach the circulation. A woman sick with dysentery gave birth to a child, which died within a few hours. Dysenteric changes were found in the intestines, and the bacillus of dysentery was cultivated from the diphtheritic deposits on the intestines, from the meconium and from the heart's blood. The organisms must have reached the child through the placenta from the circulation of the mother.

Lesions. The intestinal lesions vary from a simple inflammatory hyperemia to rather extensive superficial necrosis (diphtheritic inflammation), which

rarely extends below the submucosa. Such foci are said to be the most marked in the descending colon and sigmoid where mechanical injury is more likely to occur. The necrotic areas separate by sloughing, leaving superficial ulcers. The lymphoid follicles are swollen and infiltrated with polymorphonuclear leucocytes, which also accumulate in the dilated lymph spaces of the intestinal wall. The ileum is so commonly involved that the condition is called an ileocolitis. Conspicuous changes are not found in the mesenteric glands or spleen. The liver and kidneys commonly show parenchymatous degenerations.

Toxicity of Organisms.

The dysentery bacillus is highly toxic. Subcutaneous injections of killed cultures produce in man a more profound reaction than the organism of either cholera or typhoid. Ordinary laboratory animals are so susceptible that they are immunized with difficulty; the horse is less susceptible. The toxicity of the organism apparently depends on an intracellular toxin (an endotoxin) rather than on a soluble toxin. When living or killed cultures are submitted to autodigestion in salt solution (Conradi, Nisser and Shiga), or when bouillon cultures are allowed to grow for 30 days, the liquids are found to be toxic after the organisms are removed. In both instances this toxicity probably depends on the liberation of endotoxins. The question as to whether the bacillus in the intestines produces a soluble toxin which is absorbed by the lymphatics, is undetermined. It seems more probable that the conditions are analogous to those of cholera, intoxication resulting from the liberation of endotoxins by the solvent action of the tissue fluids or cells on the bacilli. Dysenteric symp-

toms are not produced in animals by feeding the organisms.

Dissemination and Infection.

The stools of the patient are the only known source of the organism and it continues to be excreted during convalescence. Latent or chronic cases are a source of danger to a community. Although the conditions outside the body are not favorable for the growth of the organism, it may remain living and virulent for several months. The methods of infection appear identical with those seen in typhoid. Water infection seems certain, and indirect transmission is acomplished by contact with the discharges. The best examples of contact infection are found in institutional epidemics.

Prophylaxis and Susceptibility.

The first essential for prophylaxis is correct diagnosis, for which the agglutination test and bacteriologic examination of the stools are essential. Disinfection and other precautions should be practiced as rigidly as in typhoid. The patient should not be discharged until the stools are free from dysentery bacilli.

Poorly nourished individuals are particularly susceptible to infection, and among them the mortality is high. The disease is most common among young children, old people, and those who are confined in institutions. The conditions in Japan, however, where from June to December of one year nearly 90,000 were attacked, and in Germany, where severe epidemics occur in industrial communities, indicate that susceptibility is quite general. Digestive disturbances and enteritis from other causes are said to be predisposing factors. The normal serums of man and animals have very little bactericidal power for dysentery bacilli.

Immunity.

The subject of acquired immunity to dysentery is hardly on a satisfactory basis. The serum of convalescents shows a distinct bactericidal power for the organism, and there is good reason to believe that the acquired immunity persists for some time after the disappearance of the bactericidal amboceptors, an event which takes place rather early. As in typhoid, animals which through immunization have once been stimulated to produce antibodies, form them much more readily on the occasion of a subsequent inoculation. This acquired facility in producing antibodies may be a factor in acquired immunity. By immunizing horses, serums of rather high protective power have been obtained. Kruse prepared a serum of which 1/80000 gram would save a guinea-pig from a dose of the bacilli which killed a control in 20 hours. It is assumed that the protective power of this serum is due to its bactericidal action. The antitoxic serum which Rosenthal prepared, by immunizing with 30 days' old bouillon cultures, protected not only against the toxin, but also against the bacilli; and conversely an antibacterial serum protected against the toxin (cited by Lentz). Such results leave us very much in doubt as to the existence of a true antitoxic serum.

Vaccination and Serum Therapy.

The value of protective inoculations is not well established. Shiga at one time practiced mixed active and passive immunization (bacilli plus immune serum) on 10,000 individuals. This did not decrease the number of infections, although a lower mortality resulted. Shiga claims that the therapeutic use of his serum reduces the mortality to one-third that of the untreated. The serum of Kruse, and also that of Rosenthal, are said to be cura-

tive; the discharges rapidly decrease in number and the course of the disease is shortened. In the hands of the Rockefeller Institute, antidysentery serum proved of no distinct value.

Agglutination. The agglutination reaction with the serum of patients shows great variability. It is sometimes absent in spite of the presence of bacilli in the stools, and often disappears rapidly during convalescence (in two weeks occasionally). It is rarely as high as in typhoid. In infantile diarrheas agglutinins appear at about the end of the first week of illness (Duval and Bassett). Evidently mild cases in which the course of the disease is from four to eight days may not be recognized by means of the agglutination reaction before the period of convalescence. In chronic cases the agglutinating power may persist for three or four months. No reaction was obtained with the typhoid bacillus. Kruse considers the reaction diagnostic when it occurs in a dilution of 1/50; Pfuhl, 1/30. Strong co-agglutinins for other organisms, i. e., above 1/50, have not been observed (Lentz). The tests should always be performed with both the "Shiga" and "Flexner" types, as the two have not identical agglutinable properties, and either organism may be the cause in a given instance. The absence of the reaction does not exclude a dysenteric infection positively. Bacteriologic examination of the stools is important, often necessary, for early diagnosis.

IV. MEAT POISONING BY BACILLUS ENTERITIDIS.

Botulism as a special form of meat poisoning and the occasional production of paratyphoid by infected meats, have been mentioned. In addition to these, more or less extensive epidemics,

supposed to be due to ptomains which were found in putrid meat, have occurred not infrequently. It is now well established that most epidemics of this character are caused by pathogenic bacteria which are present in the meat, whereas putrid decomposition of the latter is an unessential incident.

Bacillus Enteritidis.

Gärtner, in 1888, had the opportunity of studying an epidemic caused by the meat of a cow which had been slaughtered in extremity. The symptoms differed from those of botulism or paratyphoid, as described below. He obtained from the muscle and spleen of the cow, and from the spleen of a man who had been fatally poisoned, an organism which has since been known as *Bacillus enteritidis* (Gärtner). The same bacillus, or organisms which resemble it closely, have been obtained repeatedly during similar epidemics, both from the suspected meat and from the organs in fatal cases (intestines, blood, spleen, etc.). Drigalski, from a comparative study of several strains which had been obtained from different sources, concluded that all are members of a closely related group of organisms, the group of *Bacillus enteritidis.* His conclusions were based on cultural properties and agglutination tests.

The typical organism is a short rod, often ovoid in shape, possesses from four to twelve long flagella and has moderate motility. It ferments various sugars and is not stained by Gram's method. Variations among individual strains need not be discussed here.

Pathogenicity.

According to v. Ermengem, and also Drigalski, its pathogenicity depends on the elaboration of a soluble but heat-resistant toxin. Bouillon cultures twelve days old, in which the bacteria have

been killed by heat, also similar cultures from which the bacteria have been removed by filtration, are toxic for mice and guinea-pigs (Drigalski). It is noteworthy, however, that relatively large quantities of the bouillon were necessary to kill guinea-pigs (4.0 c.c.) which is in contrast to the toxins of diphtheria and tetanus. The rapidity with which symptoms develop following the ingestion of infected meat is a further indication of the existence of this soluble toxin, which, it would seem, is formed in considerable quantities in the meat. Symptoms occasionally develop so quickly as to suggest some strong metallic poisoning. Within a few hours vomiting, violent diarrhea and colicky pains set in, followed by more or less collapse, weakness, headache and not uncommonly by erythematous, urticarial or herpetic eruptions. Fever is absent or inconspicuous. The mortality is not high, from 2 to 5 per cent.; convalescence is said to be slow. Nephritis and catarrhal pneumonia have been noted as sequelæ. Autopsy shows the anatomic changes of an acute gastroenteritis, sometimes of hemorrhagic character, with swollen Peyer's patches; the large intestine is not greatly involved. The spleen may be swollen and the kidneys degenerated. The anatomic findings are not specific.

Sources of Infection.

It has been shown in numerous instances that the cattle or horses (Drigalski) which furnished the meat were sick with an intestinal or general infection with *Bacillus enteritidis* before they were slaughtered. "In a very large number of cases it can be demonstrated that the animals from which the meat was taken had been slaughtered in extremity or had died recently, and, indeed, that

they had (in certain instances) died before they could be slaughtered. Most often they suffer from septic inflammatory processes or from traumatic, puerperal or other sorts of septicemia, or from other ill-defined pathologic conditions which are accompanied by symptoms of enteritis or intestinal or pulmonary inflammations." (v. Ermengem.) Subsequent infection of the meat by *Bacillus enteritidis,* i. e., after slaughtering, has not been noted.

Growth in the Meat.

The organism occurs in the blood and various organs of infected animals and man. Poisoning most commonly arises when the meat has been kept for several days, which usually is the case by the time it is made into some form of sausage. In the meantime the bacilli have proliferated and additional toxin has been produced. In at least one instance a certain number of patients who ate the meat while it was fresh suffered moderate or no intoxication, whereas those who ate it several days later became violently ill. In an epidemic caused by horse meat Drigalski found that "only those persons suffered from intoxication who ate the meat after it had lain for eight days or more."

Toxin in Meat.

The micro-organism is very resistant to heat and the temperature which is attained in ordinary cooking may not be sufficient to kill the bacteria which are remote from the surface. Even in the event that the meat has been thoroughly sterilized, the heat-resistant toxin may be present in sufficient quantity to cause the intoxication. Not much is known concerning the distribution of *Bacillus enteritidis.* v. Ermengem suspects that it may be a factor in poisoning by oysters and fish, but this remains undetermined.

Agglutinins. The blood acquires specific agglutinins during the course of infection. Even eight days after the beginning of symptoms agglutination may be obtained in dilutions varying from 1/200 to 1/4000. The agglutinins disappear very rapidly. Working with artificially prepared immune serum, Drigalski determined the existence of coagglutinins for typhoid and paratyphoid bacilli.

We should bear in mind the likelihood that meats poisoned with *Bacillus enteritidis,* as well as by paratyphoid bacilli, may be encountered in America, as well as in foreign countries.

V. BACILLUS COLI COMMUNIS.

Bacillus coli communis, Bacterium coli commune, or the colon bacillus, is the type of a large group of organisms the members of which show individual differences, but possess certain dominant features in common. The typical colon bacillus ferments various sugars, with the production of gas, is a strong acid producer and curdles milk. It is flagellated, has moderate motility and does not stain with Gram's method. One type or another is the normal inhabitant of the intestinal tract of many animals, and, although the organisms are widely disseminated in nature, their occurrence is related directly or indirectly to the distribution of feces.

Resistance. Its optimum temperature for growth is 37° C., and above 46° C. it does not proliferate. It is killed at a temperature of from 60° to 61° C. in from five to fifteen minutes; it is not killed by such low temperatures as from —20° to —24° C. It resists absolute desiccation for periods varying from a few days to several months (different observers). Direct sunlight kills 99 per cent. of the

germs in two hours (Billings and Peckham), and they are very susceptible to ordinary antiseptics. The normal serums of many animals are bactericidal for the colon bacillus.

Escherich, a noted authority on this organism, lays down the principle that that strain which may be cultivated from the feces of the nursing child should be considered as the typical *Bacterium coli commune,* maintaining that a constant type of organism is found under these conditions. It is said to occur here in relatively pure culture.

Distribution in the Intestines.

Within a very short time after birth the organism is found in the intestines of infants, and its method of entrance has been the subject of much discussion. In view of its ready dissemination it is not difficult to conceive of many circumstances which favor its entrance. Having once reached the intestines, it finds there its optimum conditions for growth. The small intestines in man are rather free from colon bacilli and other organisms as well. This, perhaps, is due, to some extent, to the alkalinity of the medium and to the rather rapid flow of the intestinal contents at this point. The colon bacillus reaches its maximum development in the large intestine, where, in fact, the whole bacterial flora of the intestines is most concentrated.

Normal Functions(?)

In view of the fact that the colon bacillus is a normal inhabitant of the intestines, the conception has occurred to many that it may be of distinct value to the economy, either because of the action it has on certain foods (splitting of carbohydrates), or because in some obscure way it influences favorably the assimilation of foods, or in that it antagonizes other bacteria of distinct patho-

genic powers which also exist normally in the intestines or reach them through accident. This is not the place to consider these questions in detail, and they are on none too definite a basis. It may be stated, however, that the colon bacillus and another closely related organism, *Bacillus lactis aerogenes*, distinctly antagonize the action of certain proteolytic bacteria which appear to be associated with the putrid decomposition of milk and other proteid-containing foods. Bacteria of the latter type exist in the intestines. Unsterilized milk has a natural resistance to putrid decomposition, and sterilized milk which contains the colon bacillus or *Bacillus lactis aerogenes* has a similar resistance. These two bacteria flourish in the presence of carbohydrates, which they decompose with the liberal formation of acids, and through these acids they "limit intestinal putrefaction and influence (favorably) pathologic processes which are caused or maintained by the existing 'alkaline fermentation'" (Escherich and Pfaundler). That the organisms in question antagonize the action of putrefactive bacteria has been shown in test-tube experiments (Hirschler).

Pathogenicity. Since the time that Emmerich upheld the colon bacillus (or a colon-like microbe) as the cause of Asiatic cholera (1885), opinion as to the pathogenic powers of the organism has undergone many fluctuations. Following Koch's demonstration of the vibrio of cholera as the etiologic factor in cholera, the colon bacillus was, so to say, repressed as a pathologic agent. Later, and especially in France, great significance was again attached to it. The condition still shows a great deal of chaos, although, on account of more refined technic and the elimination of other organisms, as the dysen-

tery and paratyphoid bacilli and *Bacillus enteritidis,* from the colon group proper, we are, perhaps, on the way to a more satisfactory understanding of the pathogenicity of this organism. Although certain authors hold at the present time that the colon bacilli which normally inhabit the intestines are devoid of virulence, such a radical position is open to question. Avirulent strains have often been encountered, however.

Virulence for Animals.

As harmless as the colon bacillus appears to be when confined in the intact intestines, its virulence for animals, although low, has been demonstrated in many instances. A bouillon culture of the average bacillus which has grown for from one to two days, and when freshly cultivated from the stools, causes the death of a 300 to 400 gram guinea-pig in two or three days, when given intraperitoneally in a dose of from 2 to 3 c.c. Subcutaneous inoculations, the feeding of cultures, their introduction into the bladder and biliary passages induce inflammatory processes. It is stated (Escherich) that whether the cultures are introduced into the skin, peritoneum or vessels, symptoms of severe gastroenteritis are produced, not unlike Asiatic cholera. This fact doubtless influenced Emmerich in considering the organism as the cause of cholera. The general symptoms are those of an acute febrile intoxication.

The organism is most pathogenic when freshly cultivated, and soon loses its virulence after repeated transplantations. As in the case of some other bacteria, virulence may be re-established by "passage" through suitable animals.

The cultivation of the colon bacillus from the blood and organs of man at autopsy has not the

Virulence for Man.

significance which was once attached to it. It has been recognized that the colon bacillus in particular, and less commonly other intestinal organisms, may enter the circulation a short time before death, at a time when resistance is very low, and may obtain the general distribution which is so often encountered at autopsy; this is the so-called "agonal invasion," which may occur without much regard to the primary cause of death. The conditions which favor agonal invasion remain, to a large extent, obscure. Distinct defects of the intestinal mucosa probably are not essential, although this view has its representatives. In states of low vitality in which resistance to infection is decreased (disappearance of complement!), the organisms find conditions favorable to proliferation when they have once reached the circulation. In spite of the low virulence of the colon bacillus, it commonly has a certain amount of toxicity and it may often be of significance even as an agonal infection.

Postmortem invasion of adjacent structures, as the gall bladder and liver through the biliary passages, and of the peritoneum through the intestinal wall, also occurs.

True Infections.

It has been shown that the colon bacillus occasionally causes the following conditions: Suppurative cholecystitis which may extend to the liver, peritonitis, septicemia, meningitis, cystitis, pyelitis and ascending suppurative nephritis, and abscesses in various organs, including suppurative processes in the middle ear. In one or more instances it has been thought that it caused vegetative endocarditis. Probably colon infections of the gall bladder do not occur in the absence of biliary

stasis. Ordinarily cases of peritonitis in which the colon bacillus is encountered also show the presence of other pathogenic organisms, as streptococci or staphylococci; this is always the case in perforation peritonitis. Doubtless wrong conclusions have been drawn in many instances as to the bacteriology of peritonitis from the fact that the colon bacillus readily overgrows many other bacteria in culture media.

Cystitis.

Escherich attributes great importance to this organism as the cause of cystitis, especially in children, and states that it is probably the most common cause of cystitis, pyelitis and ascending suppurative nephritis. In 58 of 60 cases of cystitis in children the colon bacillus was found either alone or in mixed cultures. An increased agglutinating power of the patient's serum for the organism cultivated from the urine is noted in these cases.

Diarrheas.

Great interest attaches to the colon bacillus in relation to enterocolitis and dysenteric diseases. Escherich speaks of an *enteritis follicularis,* or *colitis contagiosa,* or colicolitis, epidemics of which have been noted at different times. A number of these epidemics occurred before the identification of the dysentery bacillus, and certain of them may have been true dysenteric infections. Nevertheless, dysentery bacilli are not found in all cases of enterocolitis, and the probability that genuine cases of colon enteritis occur can not as yet be neglected.

A specific colon toxin has not been obtained.

Immunization with the colon bacillus causes the formation of bactericidal amboceptors and agglutinins.

Not all strains of the colon bacillus are identical

Agglutination. in their agglutinogenic receptors. A serum which agglutinates one colon strain does not necessarily agglutinate all strains. The reaction, according to Paltauf and others, is largely an individual one. The serum of a patient with a colon infection will agglutinate the strain causing the disease, but may not affect other strains. Hence, for diagnostic purposes, the test must be performed with the culture which has been obtained from the patient. Pfaundler says in reference to colicolitis that if other colon infections can be excluded, and if the serum of the patient gives the agglutination reaction in a dilution of 1 to 50 with the bacillus which has been cultivated from the stools, colon infection is indicated (Paltauf).

VI. CHOLERA.

In 1883 Koch discovered the *Vibrio cholera* and cultivated it from the stools of cholera patients. The organism may be cultivated from the stools of the patients invariably, and is never found in other diseases nor in normal stools, except in the case of non-susceptible persons who may be encountered during an epidemic. The latter are a source of danger as "cholera carriers."

Characteristics of the Organism. Typically the cholera vibrio is about 1.5 microns long and one-fourth as broad. The cells of young cultures have the so-called comma shape which has given the organism the name of the comma bacillus. The form in reality is that of a segment of a spiral. When two cells are attached end to end an S-shape may be produced, and long spirals are made up of many cells which are joined at the ends. In old cultures the cells may assume the form of thick rods or even appear coccus-like. The vibrio possesses a single long flagellum, which is sit-

uated at the end. Although two, four and six flagella have been described, Kolle states that such organisms are vibrios of another nature. In the character and rapidity of their movement, as seen in a hanging-drop, Koch compares them to a swarm of mosquitoes. Old cultures may lose their motility to a large extent. The cholera vibrio does not form spores, although certain involution forms simulate them. It stains readily with the ordinary anilin dyes and is Gram negative.

Cultivation from the Stools.

The comma bacillus grows readily in alkaline culture media with characteristic appearances; it is an obligate aërobe under artificial conditions, in spite of the fact that it flourishes in the intestines. The optimum temperature lies between 30° and 40° C. A very simple method of obtaining the organism in pure culture from the stools was discovered by Koch. In tubes of peptone bouillon which have been inoculated with the feces of a patient, the vibrio proliferates rapidly and within a few hours exists in almost pure culture at the surface of the liquid. Isolated colonies are obtained by transferring a small amount of the surface fluid to tubes of liquefied gelatin, then plating the latter. The colonies appear in a few hours as small translucent points from which pure cultures are made on a suitable medium. For more positive identification agglutination tests are performed with anticholera serum. The Royal Institute for Infectious Diseases (Berlin) keeps on hand a dried serum of known strength (1-10,000) for this purpose. The tests being made with high dilutions, coagglutinins for other vibrios are practically eliminated. To the agglutination test may be added the "Pfeiffer experiment," in which the protective

Identification.

power of an anticholera serum is determined when guinea-pigs are infected intraperitoneally with the suspected culture. If the serum shows a protective power against this organism which approximates that shown against a known cholera vibrio, or, if the organisms are dissolved, the diagnosis of cholera is justified. In performing such experiments the serum is mixed with the culture before injection.

Resistance. The resistance of the cholera vibrio is very low. It dies in about two hours when dried (Koch) and on this account dust infection is thought not to occur. It is killed instantly by the boiling temperature, and in five minutes at 80° C. It is extremely susceptible to carbolic acid (killed by 1 per cent. in five minutes), corrosive sublimate (1 to 2,000,000 or 3,000,000 in from five to ten minutes), and to acids. Calcium chlorid is an efficient disinfectant when thoroughly mixed with the stools. The micro-organism lives in distilled water not longer than twenty-four hours, in ordinary water for several days to several weeks, and in one instance it was cultivated from the water of an aquarium after several months. Its life is short in the presence of putrefactive bacteria and rapidly-growing saprophytes, dying in sewer water in from twenty-four to thirty hours (Koch). Because of the large overgrowth of other organisms, the vibrio can rarely be cultivated from the stools later than from one to three days after death. Its life in and on foods depends on the reaction (alkalinity is favorable), and on the presence or absence of moisture. It lives longer in sterilized milk (ten days) than in that which contains other micro-organisms.

Infection Atrium and Dissemination.

Infection develops in the small intestines following ingestion of the organisms. Infection by way of the lungs or through wounds does not take place. In the patient the living vibrio occurs only in the intestines, and it is excreted only with the feces. So far as known, it has no normal habitat outside the body, although a stream or other water supply may contain the vibrio over a long period through constant reinfection of the water. This can only occur, directly or indirectly, through the stools of patients. The washing of soiled linen or bathing in water which is used for drinking and other household purposes have caused outbreaks of cholera. The water supply of a city may be infected by the discharges of patients who are confined to a ship. Convalescents may retain virulent organisms in their stools for forty-eight days (Kolle), and, as stated, healthy persons who are insusceptible to cholera and who have resided in an infected district may carry virulent vibrios in their intestines. These conditions have contributed to the futility which, to a large degree, has met attempts to limit the extension of cholera by quarantine measures. Cholera extends from country to country along the lines of travel. In some instances it has been possible to trace the origin of widespread epidemics to the delta of the Ganges, a region in which the disease is endemic. Pilgrims from India carry the infection to Mecca, and pilgrims from Egypt carry it to their native land on their return from Mecca. Either from Egypt, or through Arabia, Asia Minor and Southern Russia or Turkey, cholera has, with more or less rapidity, extended to Western Europe. The development of rapid transit has increased the rapidity with which

Epidemics.

cholera may extend. From Europe the disease has been carried to various ports of the western continent, Canada, the West Indies and southern ports of the United States, from which extension has occurred to different sections. Of six widespread epidemics of the past one hundred years, three have involved the United States, reaching considerable proportions. The means of introduction is not always apparent.

As in typhoid, two types of epidemics are known, the two often being associated: First, that caused by water infection, and, second, that in which the disease spreads by direct and indirect contact. The explosive character of an epidemic caused by infection of a water supply is much more striking than in the case of typhoid fever. In large cities hundreds, or thousands, may be striken within a day. The brief incubation period, from twelve to twenty-four hours, contributes to the acuteness of the outbreak. The distribution of a "water-borne" epidemic corresponds with the distribution of the infected water. A remarkable occurrence illustrating this point was noted in the epidemic which attacked Hamburg in 1892. In certain streets in which the residents of the two sides obtained their water supply from different sources, one of which was infected, cholera was limited to that side which was supplied with infected water. Only irregular cases due to contact infection occurred on the opposite side of the street.

Epidemics which are due solely to contact infection develop slowly and irregularly. A common incident is the successive involvement of the members of a family, whereas others in the immediate neighborhood are unaffected. Water-borne epi-

demics are invariably complicated by the occurrence of contact infection. The methods of contact infection are not different from those mentioned under typhoid fever. Food or milk which has been infected by contaminated water or by other means may cause the development of isolated groups or cases.

Susceptibility of Animals.

Animals do not contract cholera under natural conditions. By rendering the gastric contents of guinea-pigs alkaline and introducing cultures into the stomach through a tube, Koch induced a cholera-like process from which the animals died within from twenty-four to thirty-six hours; an intraperitoneal injection of opium, to quiet peristalsis, seemed to be necessary for the success of the experiment. Similar results were obtained in very young rabbits by feeding cultures to them (Issaeff and Kolle, Metchnikoff). Guinea-pigs withstand the subcutaneous inoculation of moderate amounts, but are very susceptible to intraperitoneal inoculation. Intravenous injections are exceedingly toxic for rabbits, and a fatal cholera-like condition with localization of the organisms in the intestines and intestinal mucosa has been produced in this way (Thomas).

Endotoxin.

The essential poison of the cholera vibrio is intracellular, and becomes free only after solution of the bacterial cells. Cultures which are killed carefully as by chloroform vapor (Pfeiffer) are highly toxic, although the fluid alone is non-toxic. The filtrates of young cultures have little or no poisonous action. The toxicity of older filtrates is due partly to the solution of the bacteria with consequent liberation of endotoxin, and perhaps also to secondary disintegration products which have a

certain toxicity. The soluble toxin of Metchnikoff, Roux, and Taurelli-Salimbeni is a dissolved endotoxin and not a secretion of the living cells, according to Kolle.

Conditions in the Intestines.

Koch considers that cholera is an acute infectious process of the intestinal epithelium, whereas the general condition is one of acute intoxication. It is assumed that the condition in the intestines corresponds to that in the culture media, i. e., that here, too, no true soluble toxin, comparable with that of diphtheria or tetanus, is secreted, but that the toxin which eventually reaches the circulation is that which is liberated from the bacteria after the latter have been dissolved by the bacteriolysin of the plasma, or perhaps by the leucocytes. Doubtless a great deal of endotoxin is liberated in the intestinal canal, but it is Koch's conception (cited by Kolle) that the primary intoxication comes from those organisms which have penetrated between and beneath the epithelial cells and here have undergone solution. One effect of the toxin in this situation is to cause desquamation of the intestinal epithelium, as a consequence of which rapid absorption of the toxin from the intestinal canal takes place through the denuded surface. This theory supposes that the toxin is not readily absorbed through the intact epithelium. The living vibrio has never been cultivated from the blood.

The changes in the intestines depend on the duration of the infection. In cases which prove fatal within a few hours the mucosa shows only moderate general reddening, which is intensified at the borders of Peyer's patches and the solitary follicles. The intestinal contents are of a rather

clear fluid nature in which are suspended flakes of mucus and epithelium; the fluid may be tinged with blood. With a longer duration the destructive processes in the mucosa become more intense, and consist largely of desquamation of the superficial epithelium and intense congestion of the denuded submucosa. In the more prolonged cases, "cholera-typhoid," the mucosa, especially above the ileocecal valve, may show diphtheritic necrosis. The serous surface of the intestines is injected.

Prophylaxis.

The rational prophylaxis founded by Koch, on a knowledge of the biologic characteristics of the comma bacillus, has proved of great efficiency. The essential points are the following: 1. Immediate bacteriologic examination of the stools in suspicious cases. 2. Absolute isolation of patients, in a hospital whenever possible. 3. Thorough disinfection of the stools, linen, room and all articles with which the patient has been in contact, including water-closets and privies. 4. Continued isolation during convalescence until the stools are free from vibrios. 5. Repeated bacteriologic examination of the stools of those who have been in contact with cholera patients until their freedom from vibrios is assured. 6. Frequent examination of the water supply at different points in order to detect the occurrence of water infection. 7. In case water infection exists, exclusion of the water from all domestic uses, and the institution of means to rid the water of infection. This may be done in the case of infected wells, but in the case of large systems reconstruction may be necessary for future protection. Water for household use should be boiled. Kolle compares the conditions in Germany and Russia during the epidemic of

1892-4. In Germany, where Koch's principles of prophylaxis were rigidly observed, about 10,000 cases occurred, 9,000 of which were confined to Hamburg, whereas in Russia, where precautions were not enforced strictly or generally, 800,000 cases developed during the same period.

Vaccination. Protective inoculation has shown itself to be of distinct value for prophylaxis. Ferran, a Spaniard, first practiced vaccination on a large scale in 1884, although little definite knowledge of the value of the procedure resulted from his work. He is supposed to have used impure cultures. Haffkine introduced protective inoculation on a large scale in India, and up to 1895 had inoculated 40,000 persons. Following Pasteur's method with anthrax, he used two vaccines. Vaccine 1 was a culture which had been attenuated by prolonged growth at 39° C. Vaccine 2, which was administered five days later, was a virulent culture. The living organisms were used in both vaccines and the injections were given subcutaneously. The local and general symptoms were mild. Instead of living cultures Kolle has proposed the use of virulent cultures which have been killed by exposure to a temperature of 58° C. for one hour. The vaccine is preserved by the addition of 0.5 per cent. phenol. In the Japanese epidemic of 1902 this method was used on an extensive scale. The incidence of disease among the uninoculated was 13 per cent., among the inoculated 0.06 per cent.; the mortality among the uninoculated was 10 per cent., among the inoculated only 0.02 per cent. The disease, when it occurred in the inoculated, was of a mild type. A single injection of from 2 to 4 mg. of a killed agar growth was given sub-

cutaneously (cited by Kolle). Strong has proposed the use of the products of autolysis of the cholera vibrio as a vaccinating substance, a method founded on the observations of Neisser and Shiga in relation to typhoid, and of Conradi and Drigalski in relation to dysentery. The local and general symptoms are said to be of a mild type. The method has had no practical trial.

Natural Immunity and Susceptibility.

From what was said above in connection with the so-called cholera carriers, it is evident that not all are equally susceptible to infection with cholera. In the few instances in which infection has been attempted deliberately, some contracted the disease, at least one case ending fatally, whereas in others either a mild infection or none at all took place. The conditions on which such cases of individual immunity depend are not known conclusively, although it is often intimated in a general way that a strong bactericidal power of the body fluids, or a high phagocytic power on the part of leucocytes, is responsible for it. The gastric juice, on account of its acidity, offers a barrier to the passage of living vibrios into the small intestines, and this is particularly true of cholera. It is nevertheless evident that the barrier in many instances is not a serious one. A number of cases are recorded in which investigators while working with cultures have become infected with cholera, the cases running typical courses which sometimes ended fatally (Pfeiffer, Pfuhl and others). Organisms which are ingested with water may pass rapidly to the intestines without being affected by the acid of the stomach, or when taken with food they may be buried in the latter and hence not come in contact with the gastric secretion. It

seems probable that the intestinal epithelium has a certain resistance to invasion which is most manifest in the case of those who do not become infected in spite of the presence of the organisms in their intestines. Natural immunity appears to be one which is directed against the bacteria rather than against the endotoxin, proliferation of the organisms in the intestinal epithelium being prevented. Poorly nourished individuals, the very young and the very old are particularly susceptible. Other gastrointestinal disorders, in the presence of an epidemic, predispose to infection. Defects in the intestinal epithelium, or a decreased resistance of the latter (!), may afford favorable conditions for invasion.

Acquired Immunity.

Active immunity, as that which results from infection or from protective inoculation, is characterized by the appearance of bactericidal amboceptors, agglutinins and specific precipitins in the serum. It is now widely believed that acquired immunity depends on the presence of the bactericidal amboceptors in the circulation and body fluids, although Metchnikoff holds, on the other hand, that it depends largely on an increased phagocytic power on the part of the leucocytes. According to Pfeiffer and Marx, the antibodies are produced in the blood-forming organs. An attack of cholera confers immunity of prolonged duration, although it is not always absolute.

Passive immunity is readily induced in animals by the injection of anticholera serum. As in other instances, it is of short duration. Doubtless the same condition may be induced in man. Besredka has proposed mixed active and passive immunization for protective inoculation, using killed bac-

teria which have been saturated with the specific amboceptors.

Serum therapy has been no more successful in cholera than in typhoid fever. The antitoxic serum of Roux and others has had no practical trial. According to Achard and Bensaude, the serum of cholera patients, on the third or fourth day of the disease, agglutinates the cholera vibrio. However, they used the serum in dilutions of 1-20, and in this strength even normal human serum may be agglutinating (Pfeiffer and Kolle, cited by Paltauf). Convalescents even after seven months may show an agglutinating power of from 1/100 to 1/120. **Serum Therapy and Agglutination.**

The bacteriologic examination of the stools is the most reliable means of early diagnosis (see above).

VII. PLAGUE.

Plague was known in the second and third centuries. In the sixth century it ravaged the Roman empire and destroyed half the population in the eastern provinces. Under the name of the "black death" it swept over Europe in 1347-50 with a sacrifice of one-fourth of the inhabitants—about 25,000,000. During the fifteenth and sixteenth centuries many epidemics prevailed in various parts of Europe, and the disease seemed to have fastened itself on that part of the world. However, the pneumonic form of the disease, the most contagious, gradually became less common, or the virulence of the infection diminished, and this, with the institution of quarantine regulations, decreased the prevalence of the plague during and following the seventeenth century. Nevertheless, there have been occasional outbreaks in Eastern Europe since

that time. Following the recrudescence of plague in Hongkong in 1893 and in other places later, the disease has been subjected to scientific study, its cause has been discovered, and the importance of rigid quarantine measures at seaports in preventing its universal extension has been proved.

Characteristics of the Organism.

In the Hongkong epidemic of 1893-4 Kitasato and Yersin, working independently, discovered the bacillus of plague, *Bacillus pestis*. The organism is minute (1.5 to 1.75 by 0.5 to 0.7 microns), and typically is of long oval shape. The frequent occurrence of short oval cells (coccus form), longer rods and distorted, swollen, vacuole-like cells (involution or degeneration forms) signifies a high degree of pleomorphism which is characteristic. The longer the disease has lasted, or, on the other hand, the older the culture, the more numerous are the atypical forms. In bouillon long chains develop. It is non-motile, has no flagella and forms no spores. A capsule may be demonstrated by appropriate technic. It does not stain by Gram's method, and with methylene blue, carbol fuchsin, etc., the ends stain more densely than the central portion (polar staining). Because of its general properties it is placed in a group with a number of bacteria which cause hemorrhagic septicemias in various animals—the "hemorrhagic septicemia group."

There occurs in bouillon the so-called "stalactite" growth, in which visible processes extend from the surface toward the bottom, where they meet other processes which extend toward the surface ("stalagmites"). These formations utilize as their starting points the side of the flask or drops of butter or oil which are placed on the surface. Cer-

tain other organisms grow in a similar manner. It is said to be a characteristic feature of the plague bacilli that many involution forms appear on agar which contains 3 per cent. of sodium chlorid. The optimum temperature for growth is from 25° to 30° C., which is somewhat lower than that for most pathogenic organisms. It grows rather slowly even under the best conditions. In mixed cultures it is overgrown by saprophytic organisms (e. g., colon bacillus).

Viability and Resistance.

The plague bacillus may live for from four to seven days in the putrefying organs of man or animals. Its virulence may be retained in the cadaver of a rat for two months (Bandi and Stagnitta-Balistreri). During this time the organisms penetrate all the tissues of the body, even growing through the skin. It may live in the pus of a bubo for twenty days when unmixed with other organisms (Albrecht and Gohn); in the sputum from plague pneumonia for ten days; in various foods, as milk, potatoes, for one to three weeks; in water from five to twenty days, depending on the number of saprophytes which are present; in earth from two weeks to three months, depending on the quantity of organic matter and other organisms. In all these instances the higher the temperature, i. e., above 30° C., and the more numerous the saprophytic organisms, the shorter is the life of the plague bacillus. In winter, when contaminating saprophytes grow less rapidly, the plague bacillus lives longer. Its resistance to desiccation, sunlight and disinfecting agents is rather low, particularly when the surrounding temperature is above 30° C. In temperatures of from 29° to 31° C., when thoroughly dried, it rarely lives longer

than from six to seven days, whereas at lower temperatures, 16° to 20° C., cultures may be obtained after from one to several weeks, depending on the material which contains the organisms. It lives longer in woolen and cotton threads (clothing) than when isolated as in dust; hence, dust infection is improbable (Dieudonné). In sputum (plague pneumonia) and purulent exudates in which the bacilli become incrusted to a degree, life may persist for from three to four weeks. Sunlight kills them in from two to six hours, depending on the temperature and the proximity of the organisms to the surface. Although cultures for the purpose of vaccination have been killed at a temperature of 65° C. for one hour, precautions to insure an even distribution of the heat are necessary to render certain the death of all organisms. A temperature of 100° C. kills them at once, and 80° C. in from five to ten minutes (moist heat). They are very resistant to cold, remaining alive at a temperature of —20° C. for several weeks, even when repeatedly thawed out during this time, and they even proliferate slowly at from 4° to 7° C.

Virulence and Toxins.

Cultures of the plague bacillus retain their virulence over a long period when kept in a cool dark place and when not allowed to dry. However, they often loose in virulence unaccountably. The nature of the toxic substance is as yet obscure. A concentrated soluble toxin has never been obtained in cultures. Filtrates of young cultures show little or no toxicity, whereas in older cultures the fluid becomes more or less toxic (liberation of endotoxin?). Lustig and Galeotti extract cultures with 0.75 to 1 per cent. potassium hydroxid, from which they precipitate a toxic substance with ace-

tic or hydrochloric acid. Markl found the cell bodies to be very toxic after eight weeks' growth at room temperature, provided the organisms were killed by chloroform rather than by heat; killing by heat destroys the toxic substance largely. He believes some metabolic product of the organism is the chief toxic constituent, claiming at the same time the presence of a certain amount of soluble toxin.

Virulence for Animals.

The plague bacillus is exceedingly virulent for rats, guinea-pigs and monkeys; somewhat less virulent for mice and adult rabbits; other animals, cats, dogs, swine, cows, horses, sheep, goats, may be infected artificially, although they commonly recover even after large doses. Rats and guinea-pigs may be infected by subcutaneous, intraperitoneal and intravascular injections, by the feeding of infected material or by placing it on the nasal mucous membrane or in the conjunctival sac, and by inhalation experiments, the last method commonly resulting in plague pneumonia. Guinea-pigs and young rabbits die of plague septicemia in from four to five days when cultures or material containing the organisms (sputum, feces, organs from plague cases), are rubbed into the shaven or even unshaven skin (Albrecht and Gohn). This experiment is of value for detecting virulent plague bacilli and separating them from contaminating organisms. Following inoculation into a cutaneous or mucous surface a local reaction of varying intensity develops in which the subcutaneous tissue becomes edematous or even hemorrhagic, in a number of hours the regional lymph glands become swollen and hemorrhagic, and in from two to five days the animals die of plague septicemia. Cul-

tures of low virulence not infrequently cause a chronic infection which is characterized by the formation of large granulomatous nodules on the surface of the liver and spleen and in the omentum. Such foci contain many plague bacilli, and the death of the animal results in a few weeks from intoxication or from general infection. Although rabbits are much less susceptible than rats or guinea-pigs, young animals succumb to cutaneous inoculation.

Endemic Plague.

Dieudonné cites four foci in which plague is known to be endemic at the present time: One is in China (province of Yünnan), from which the Hongkong epidemic originated; a second in the Himalayas, which led to the outbreak in Bombay; a third in a mountainous region south of Mecca, and a fourth was found by Koch and Zupitza in British East Africa near the source of the White Nile.

Plague in Rats.

The opinion is held by many that plague is primarily a disease of the rat and that certain regions remain pest-infected because of this fact. Rats, in certain districts, suffer from a chronic form of the disease, and it is possible that the organism at times acquires increased virulence, as a consequence of which the infection becomes widespread and rapidly fatal among these animals. It is probable that the chief method of transmission from rat to rat is through the eating of plague cadavers. The possibility of transmission from one animal to another by means of fleas is upheld by some. The blood which fleas suck from infected rats frequently contains bacilli, but transmission to other rats by the bites of fleas is still disputed.

The means by which the disease extends from

rat to man are not definitely determined. This much is known, however: First, that the bacilli are excreted in the urine and feces of infected animals, and, second, that the disease attacks those especially who live in dark, damp, filthy quarters in which rats are numerous. Europeans who live under hygenic conditions in plague-infected districts rarely contract the disease. A great mortality among the rats not uncommonly precedes an outbreak of plague in man. The existence of "plague houses" may depend on the prevalence of the disease among the rats which infest the houses. On the other hand, the organisms excreted by a plague patient through the sputum, urine or feces, find, in the conditions described above, surroundings which favor their prolonged life; hence, the occurrence of subsequent infection in the same house in many instances may be traceable to a previous case.

Plague Houses.

The theory has been advanced also that fleas may be an important means of transferring plague from rats to man. This is objected to on the ground that every animal has its peculiar flea and that the flea of the rat will not bite man. Nevertheless, it may alight on the skin of man temporarily and there discharge bacillus-laden excretions. Flies, in a like manner, may distribute the bacilli from rats or the infected excretions of man.

Fleas.

When plague invades a new country it commonly makes its first appearance in coast cities. Presumably this is accomplished through infected rats which may board a ship during its stay in a pest-ridden harbor, and which subsequently escape at the new port.

Epidemics of plague lack the explosive-like sud-

Epidemics. denness in their development which characterizes cholera and, to a certain extent, typhoid and dysentery. The cases occur in groups and in particular houses in such a manner that direct and indirect contact seem to be largely responsible for transmission. Every epidemic of plague may be divided into three stages: a slow progression from small centers, an acme of widespread death, and a slow recession (Dieudonné). It seems probable that the disease spreads rapidly and extensively only when the pneumonic form prevails.

Infection Atria. In man infection takes place through the skin most frequently, although the mucous membranes of the mouth, nose, pharynx, tonsils or the conjunctiva are possible infection atria. Often no local reaction is produced, and the point of entrance may be indicated only in a general way by the swollen lymph glands of the region. Infrequently a pustule or small carbuncle marks the point of entrance. Primary plague pneumonia is caused by the inhalation of pest-laden material, particularly fine particles of sputum from a pneumonic case, and perhaps also by the inhalation of infected dust; the latter is probably of less importance because of the short life of the organism in dust. Even in ordinary speaking minute drops of saliva are thrown into the air. Infection is thought not to occur through the stomach or intestines. In the pneumonic and septicemic forms, the infected urine and feces contribute to the dissemination of the organisms. Transmission by indirect contact, as by infected clothing and linen, has been noted in many instances. Compared with pneumonic and septicemic plague the bubonic form is much less dangerous to a community.

Following cutaneous infection the regional lymph glands become swollen and hemorrhagic, and undergo more or less extensive necrosis. When the infection extends beyond the lymph glands the blood may contain enormous quantities of bacilli (plague septicemia), and the same condition follows plague pneumonia; in the event of general infection death follows in a few hours. "Secondary pneumonia" and also "secondary buboes" develop as a consequence of blood infection. Hemorrhages into the mucous membrane (especially the stomach or cecum), endothelial surfaces (pericardium), and various parenchymatous organs, with extreme degeneration of the latter (liver, kidneys and heart), are characteristic anatomic changes. The spleen is usually swollen. The toxic substance evidently has affinities for many tissues.

Mixed infection with the streptococcus is not uncommon and is a serious complication.

Prophylaxis.

The following are important points for prophylaxis: 1. Early diagnosis as established by bacteriologic examination of blood, sputum, and fluid taken from a bubo either by a syringe or after incision; 2, in the thorough isolation of patients and of those whose have been exposed to infection; 3, in the disinfection of excretions, of clothing and of infected houses, which in some instances may mean the destruction of the latter; 4, in the destruction of rats; 5, prophylactic injections. Up to the present time the most effective measure of getting rid of rats is to offer a bounty for each animal caught, as practiced in Manila.

Vaccines

The vaccine of Haffkine has been used extensively in India. The Indian plague commission found that the incidence of disease and the mor-

tality were lower among the inoculated than the uninoculated, although many of the inoculated contracted the disease in a benign form. The vaccine consists of bouillon cultures which have grown for six weeks with stalactite formation (see above), then killed by exposure to a temperature of 65° C. for one hour; from 0.5 to 3.5 c.c. are injected, according to the age and size of the individual. One or more subsequent injections may be given. The local and general reactions are of moderate severity. Protection becomes manifest only several days after the inoculation and may persist for many weeks or months. The vaccine recommended by the German commission consists of two days' old agar cultures which have been killed by heat (65° C. for one hour). Lustig and Galeotti utilize the toxic precipitate described above as a vaccine. Terni and Bandi inoculate rabbits or guinea-pigs intraperitoneally with the plague bacillus and after or just preceding death collect the peritoneal exudate, in which the organisms are allowed to proliferate still further for twelve hours. The bacilli are then killed at a low temperature, and this fluid, after an addition of a preservative, constitutes their vaccine. Although the last three vaccines have proved of value in animal experiments, they have not as yet been used extensively in man.

Besredka, also Shiga, recommend the use of mixed active and passive immunization, as suggested in relation to typhoid and cholera, in this instance naturally using plague bacilli (killed) and anti-plague serum. Shiga reported good results by the use of the combined method in the epidemic in Kobe.

The immunity which is produced by protective

Immunity.

inoculation, like that which follows natural infection, is considered to be antibacterial inasmuch as the serum acquires increased bactericidal power for the bacillus, but shows no ability to neutralize its toxic constituents. As in relation to many other infections, however, we are not in position to ignore the possibility of an increased phagocytic power on the part of the leucocytes. The influence of opsonins is essential for experimental phagocytosis. Wright characterizes the plague bacillus as "an organism which is absolutely insensible to the bactericidal action of the normal human blood fluids, but eminently sensible to the opsonic action of these fluids." The immunity which follows infection is of long duration.

Serumtherapy and Prophylaxis.

Prophylactic injections of antiplague serum produce a temporary immunity of about two weeks' duration. The Pasteur Institute prepares the serum of Yersin by immunizing horses first with killed and then with living cultures. The immunization is difficult and from several months to a year and a half are required for the production of a strong serum. When the blood is drawn eventually its freedom from living plague bacilli and from toxic substances must be assured. The immunizing value of the serum is determined by that quantity which will save a mouse from a fatal dose of living plague bacilli, the serum being given twenty-four hours in advance of the culture. This is accomplished by 0.1 to 0.02 c.c., depending on the strength of the serum. Its curative power is estimated from that quantity, 0.5 to 0.1 c.c., which saves a mouse when administered sixteen hours after the injection of an otherwise fatal dose of culture. For protective inoculation in man

from 10 to 20 c.c. are recommended, and for curative purposes from 30 to 50 c.c. Concerning the value of this serum Dieudonné concludes as follows: "On the basis of the results obtained in man and in animal experiments we can attribute no positive curative value to the Parisian serum, although a certain influence on the course of the disease can not be denied. On the other hand, the serum is suitable for protective inoculation when immediate immunity is necessary, as for those who are caring for cases of plague pneumonia. Since, however, the protection afforded by this means persists only for a few days, subsequent active immunization with killed cultures is indicated as soon as possible for those persons who are exposed to infection for some time." The favorable results noted by a number of observers would seem to justify further use of the serum for curative purposes.

The serum of Tavel, prepared at the Institute of Bern, is, like that of Yersin, bactericidal and agglutinating. Antitoxic as well as bactericidal properties are claimed for the serum of Lustig, which is prepared by immunization with the toxic precipitate mentioned above. It has been used extensively in the treatment of plague and in a number of small epidemics favorable though not thoroughly convincing results were reported. The serum of Markl, which is supposed to be antitoxic, has had no practical trial. It is prepared by immunization with old cultures which have been killed by chloroform.

Agglutination. Although the serum of patients acquires a certain agglutinating power, it is rather low (1/3 or 1/5), and does not become manifest until during

the second week of the disease. Before this time diagnosis by clinical or bacteriologic means can be made with certainty; hence, for clinical diagnosis the reaction has little value. On the other hand, a strong artificial agglutinating serum obtained by the specific immunization of animals is of great value for the identification of the plague bacillus when cultures have been obtained from suspected cases. Artificial serums may agglutinate in dilutions of from 1/1000 to 1/6000.

B. Serum in acquired immunity is not bactericidal, or knowledge on this point is deficient.

I. ANTHRAX.

From the standpoint of infection and immunity anthrax is of particular interest. It is the first disease of which the bacterial etiology was proved and in which the specific microbe was used in pure culture for the production of artificial immunity (vaccination).

Anthrax is particularly a disease of cattle and sheep, and it prevails in certain European countries, especially Russia, in Australia and in South America. It does not occur extensively in this country. Definite regions are at times heavily infected, and it is in such localities that the disease is most frequently transmitted to man.

Bacillus Anthracis.

As early as 1850 Rayer and Devaine, also Pollender, had discovered the presence of small rods and filaments in the blood of animals which had died of anthrax, and the work of Koch, Pasteur and others soon established that this rod, the anthrax bacillus, is the cause of anthrax. The discovery of Koch that the bacillus forms extremely re-

sistant spores, explained the persistence with which the disease infects particular localities.

Spores. The anthrax bacillus is a fairly large organism, is rod-shaped, non-motile and grows with characteristic appearances on various culture media. With the proper temperature and culture medium, and in the presence of free oxygen, the formation of spores begins after about twenty-four hours of growth. Their evolution is complete in from one to two days, and eventually the protoplasm of the cells disintegrates and the spores are set free. Spores are not formed in the body of an infected animal. Spore formation is not essential, however, for the continued life of the organism; at high temperatures (42° C.), and in the presence of minute amounts of acids and alkalis or of carbolic acid, strains may be so altered that they lose permanently the ability to produce spores. Under favorable conditions the spores germinate completely in from three-quarters to one and one-half hours (Grethe) by a process in which they lose their refractive appearance and assume first an oval and then a rod shape. In the body a capsule surrounds the bacillus, and it grows singly or in very short chains; in culture media it is very difficult to obtain capsules. The long threads which appear in culture media, especially bouillon, are not found in infected animals.

Resistance and Virulence. The bacillus itself shows no unusual resistance, but its spores are more resistant than those of any other pathogenic bacterium. When dried on a thread they have been known to live for from ten to twelve years. Corrosive sublimate (1-2000) kills them in forty minutes (Fraenkel), and direct sunlight in about 100 hours (Momont). *Bacillus*

pyocyaneus, streptococci, staphylococci and the bacillus of Friedlander are said to antagonize its growth, and Rettger found that the dried *B. prodigiosus* decreased the virulence of the organism for animals when the two were injected.

The anthrax bacillus is remarkable for its infectiousness. A twenty-millionth of a loop of a virulent culture will cause a fatal infection in mice, guinea-pigs and rabbits, when given subcutaneously. A systemic infection may be produced by feeding the spores or causing animals to inhale them. The gastric juice is able to kill the bacilli, but not the spores, which germinate after they reach the intestines.

The organism is distributed by the excretions of diseased animals, and after their death the adjacent soil becomes heavily infected by the discharges which escape from the intestines and bladder. In this situation the bacilli pass into the sporing stage, in which they remain viable and virulent for a long time.

Infection Atria.

The infection of herds usually is accomplished by the ingestion of spores which have been distributed in this way, the spores germinating, as described above, after they have reached the intestines. The disease may be primary in the skin in the form of malignant pustule. In man malignant pustule is the commonest type of infection, occurring especially among those who have to do with cattle and sheep. The bacilli, however, may gain entrance through the lungs as in the so-called "wool-sorter's" disease, which is caused by the inhalation of infected dust from the raw material.

The generalized infection in all animals is rapidly fatal (one to three days), and the occurrence of

death is sometimes so sudden as to be called apoplectiform; in man the mortality is about 50 per cent. Malignant pustule runs a more favorable course.

Toxin. The general infections are marked by symptoms of intense intoxication and acute degenerative changes are produced in the parenchymatous organs. Massive numbers of the bacilli are found in the blood. Neither a soluble toxin nor an endotoxin characteristic for the organism has been demonstrated up to the present time (Sobernheim), although there is abundant clinical and anatomic evidence of intense intoxication. The production of mechanical injuries by the large masses of bacilli in the circulation is doubtful.

Prophylaxis Rational prophylaxis involves the proper disposal of the bodies of animals which have died of anthrax, the exclusion of animals from fields known to be infected, suitable disinfection of stalls, and finally protective inoculation against the disease. No part of the anthrax cadaver should be used for commercial purposes, because of the danger of infecting those who work with the raw materials. Cleanliness and the usual precautions against contagious diseases should be observed by those who are exposed to infection, bearing in mind that the disease may be transmitted by way of the lungs and alimentary tract, as well as by the skin.

Natural Immunity and Susceptibility. It is probable that no disease is more perplexing from the standpoint of immunity than anthrax. The variations in susceptibility and immunity among different animals are extreme: Guinea-pigs, rabbits and mice are probably more susceptible than sheep and cattle; compared with these the dog and rat are relatively immune, whereas fowls

and cold-blooded animals can be infected with difficulty. Although the microbe is readily killed by suitable serums (rabbit, e. g.), such an effect is not an index of immunity. The serum of the highly susceptible rabbit is strongly bactericidal in test-glass experiments, whereas that of the more resistant dog, or rat, has little or no bactericidal power. Because of this inconsistent relationship of the serum to immunity, and since the leucocytes have a high phagocytic power for the anthrax bacillus, Ptruschky, Frank and others agree with Metchnikoff in assigning variations in the natural immunity of different animals to variations in phagocytic power. Bail and Pettersson, in extensive experimental work, discovered conditions which, they believe, explain the lack of correspondence between serum properties and natural immunity. In the serum of the relatively immune dog and chicken they found bactericidal amboceptors but no complement; hence, the serum could show no bactericidal action in the test-glass. If, however, leucocytes from the same animals were added to the serum, the latter became bactericidal. It may be assumed that in the course of infection the amboceptors are activated by complement which is discharged from the leucocytes. The failure of the bactericidal substances of the rabbit's serum to protect the animal was ascribed to the ability of the tissues to absorb the amboceptors (cited from Sobernheim). Their work is of sufficient importance to demand repetition.

Wright has shown the importance of the opsonins for phagocytosis of the anthrax bacillus.

Recovery from spontaneous infection is said to

confer a degree of immunity, which, however, is not permanent.

Vaccination. Artificial immunity may be produced by active or passive immunization. The first attempts at vaccination were made in 1880 by Toussaint, who injected the blood of infected animals after it had been heated to 55 degrees for ten minutes. The bacilli were thus attenuated, but they were able to form spores subsequently and vaccination was not always successful. Pasteur used two vaccines. Vaccine I consisted of a culture which was attenuated by growth at 42° C., and which contained no spores. Vaccine II was a virulent culture, and was injected in from ten to fourteen days after vaccine I. Its use is said to have caused a decrease in anthrax in heavily infected districts, with a consequent decrease of the disease in man. Various modifications of the vaccines of Pasteur have been devised by others, and they seem to be equally successful. In some instances killed bacilli and the products of bacterial growth have been used with less success. The *Anthracase-Immunproteidin* of Emmerich and Löwe is not of established value.

Serum Therapy and Prophylaxis. Immune serum for therapeutic purposes is prepared by immunization, first with killed or attenuated cultures and then with virulent strains. The two vaccines of Pasteur may be used. Although the serum has been shown to have fairly strong protective powers, it is of less value when used for curative purposes. It produces no effect after the blood stream has been invaded by the bacilli. Its greatest value is for the protection of herds when anthrax has declared itself. In man it has been used chiefly in the treatment of malignant pustule in which the prognosis, even without specific treat-

ment, is not unfavorable. The best known serums are those of Sclavo, prepared from the goat and ass, of Mendez and Deutsch. The properties on which the value of the serums depends are unknown. Sobernheim is very positive in stating that the bactericidal power of an animal's serum is not increased by immunization or infection, and the existence of an antitoxin is not recognized. As in some other instances immunization may cause an increase in opsonins which would render the serum effective by its power to cause increased phagocytosis.

Mixed Immunization and Agglutination.

The method of Sobernheim, that of mixed active and passive immunization, seems to be successful as a prophylactic measure. The vaccine consists of a mixture of antiserum and bacilli. Immune and even normal serums at times may agglutinate the anthrax bacillus, but the reaction is inconstant, and the ability of an immune serum to cause agglutination is no index of its protective power. Agglutination is somewhat difficult of determination because of the tendency of the bacillus to grow in the form of chains.

II. MALTA FEVER.

Malta, Mediterranean or undulant fever, discovered in the Island of Malta, is also seen among British troops at Gibraltar, and cases have been discovered in the Caribbean Sea, Porto Rico, in Hongkong and in Manila. Historically, it has been traced to the beginning of the nineteenth century, but it was first described as an independent disease by Marsten in 1859. It is said to be extending. The disease usually runs a long course, which is somewhat typhoidal in character, and there may be one or

more relapses. The spleen is enlarged, but the intestines are not involved.

"It is distinguished from typhoid by its long duration, sometimes extending over many months; by a course of fever exhibiting marked undulations; by the occurrence of copious perspirations; by the frequent appearance of rheumatic articular disorders as well as by neuralgia and inflammation of the scrotum and epididymis" (Scheube). It occurs especially in the summer months.

Basset-Smith found the serum in practically all stages of the disease and in convalescence to have little or no bactericidal power for the coccus. Normal serum appeared to be more bactericidal than that of the patients, although such an action was often missed in normal serum. Wright says that normal human serum is devoid of bactericidal power for the organism. Basset-Smith also concluded that the phagocytic power of the patient's leucocytes is less than in the case of normal leucocytes. According to Wright, the organism "is eminently sensible to the opsonic action of the normal serum," under the influence of which it is taken up in large numbers by the leucocytes.

Agglutination by the serums of patients takes place in dilutions varying from 1-300 to 1-2000 or even as high as 1-6000. Agglutinins develop fairly early in the course of the infection, and the test is of great diagnostic importance.

Bacillus melitensis, discovered by Bruce (1887) in the spleen of patients who had died of the disease, is a minute organism, slightly oval in shape. According to Gordon, it possesses one flagellum, rarely two or four, and is slightly motile. The bacillus is found in pure cultures in the spleen,

which is greatly enlarged. Its growth in culture media is very slow.

It is thought that infected water may be one means of transmission of the disease. Laboratory infections have occurred through small wounds. The disease is not transmitted from person to person.

Up to the present time the monkey is the only animal known to be susceptible to artificial infection, although the organism may have a low degree of virulence for rabbits and guinea-pigs (Durham).

One attack confers immunity, which may disappear, however, after some time (Hughes).

An immune serum which was prepared by Wright is said to influence favorably the course of the disease.

GROUP III.

Acute infectious diseases in which acquired immunity of prolonged duration is not established. In some instances soluble toxins are produced which are of unknown importance in infection (staphylococcus, streptococcus). Some of the organisms contain rather strong endotoxins (pneumococcus, gonococcus), whereas in others a reasonable basis for their infectiousness is not at hand. In some instances immunization causes increased resistance to infection (staphylococcus, streptococcus), whereas this property has not been fully demonstrated in others.* The serums of immunized animals may or may not be protective for other animals. Those organisms which cause systemic infection give rise to clinical leucocytosis (except influenza). Local inflammations are accompanied by the accumulation of polymorphonuclear leucocytes.

I. PNEUMOCOCCUS INFECTIONS—PNEUMONIA.

Organisms Causing Pneumonia.

No one organism is the exclusive cause of any one type of pneumonia, except perhaps the viruses of syphilis and tuberculosis. Any microbe which causes pneumonia can also set up inflammations in other organs. The following may cause acute pulmonitis: *Diplococcus pneumoniæ, Streptococcus pyogenes, Staphylococcus pyogenes,* bacillus of Friedlander (*B. pneumoniæ*), *B. influenzæ, B. pestis, B. diphtheriæ, B. typhosus, B. coli communis,*

* This point is difficult of determination when an organism has little or no pathogenicity for animals (influenza, gonococcus, bacillus of Ducrey, etc.).

and *Micrococcus catarrhalis.* The organisms of tuberculosis, actinomycosis, the virus of syphilis and some other infections cause chronic inflammations of the lungs. Some of these organisms have already been considered and others will be discussed later, in their relation to pneumonia, without, however, entering into details as to the various types of the disease. The *Diplococcus pneumoniæ* is the commonest cause of lobar pneumonia. It produces lobular pneumonia not infrequently, and has been found as the only organism in acute interstitial pneumonia (Weichselbaum).

Diplococcus Pneumoniæ.

Friedlander (1882) found that capsulated cocci were present constantly in the exudate of pneumonia. Such cocci in all probability represented the organism which at present is known as the diplococcus of pneumonia, yet the cultures which he obtained somewhat later showed the characteristics of the organism now known as the bacillus of Friedlander. Fraenkel, in 1884, obtained the first-named coccus in pure culture, and his investigations, together with those of Weichselbaum and many others, eventually established the independence of the two organisms.

Typical and Non-Typical Strains.

The typical pneumococcus is slightly elongated, and both in the tissues and in culture media it grows in pairs. Typically, also, the pair possesses a capsule which is present constantly in the tissues and may be obtained on certain culture media (milk and serum). It is non-motile, non-flagellated, forms no spores and stains by Gram's method. Rather scant growth occurs on the ordinary culture media in the form of small colonies which resemble those of the streptococcus, and unless special media are used it usually can not be carried

through many generations. When grown in sputum, or on a medium which contains ascitic fluid, the blood or serum of man or some favorable animal, its virulence may be preserved for some time. By growth at 39° C. virulence is lost rapidly. Strains which are atypical in one of several ways are encountered. They may show low virulence, may grow well at ordinary temperatures (the typical organism not doing so), may produce long chains in liquid media, or may grow without a capsule.

Confusion with Streptococcus.

Recently the danger of confusing the pneumococcus with the streptococcus has received renewed attention, and newer methods of differentiation render it extremely probable that such confusion has occurred in the past. An important differential method is that of cultivation on agar plates which contain blood (Schottmüller); the streptococcus produces a clear zone of hemolyzed corpuscles about its colonies, whereas the colonies of the pneumococcus present a greenish color and produce no hemolysis. In using this test Ruediger found a surprising number of pneumococci in normal throats, whereas previous work had shown them to be much less common than streptococci.

Resistance.

In spite of the poor viability of the organism on ordinary culture media, it is fairly resistant to desiccation and sunlight, especially when imbedded in sputum. It is possible that the surrounding sputum is protective and that the well-formed capsule which the coccus possesses as a parasite, increases its resistance. When dried and powdered it is much less resistant, being killed by direct sunlight in about an hour. Like other bacteria, it resists diffuse sunlight better than direct, and in the former

may live for as long as fifty-five days in a dried state (Bordoni-Uffreduzzi, cited by Weichselbaum). It has very little resistance to heat, being killed by a temperature of 52° C. for ten minutes.

Toxic Properties.

No characteristic soluble toxin has been obtained, although more or less poisonous substances, some of them of a chemical nature, have been described. Presumably the toxic properties reside in an endotoxin. The pneumotoxin of F. and G. Klemperer was prepared by precipitation with alcohol. The pneumococcus is a pyogenic organism and causes exudates which are rich in fibrin. Occasionally serous rather than purulent exudates are produced. Its toxic action is directed toward various organs, and it is doubtful if any of the tissues of the body are non-susceptible. Some strains are supposed to be more neurotoxic than others.

Susceptibility of Animals.

The susceptibility of animals varies greatly. Rabbits and mice are extremely susceptible and are used as test animals for the identification of the organism. Other laboratory animals have greater resistance, and the pigeon and chicken are almost absolutely immune. In susceptible animals a rapidly fatal coccemia or more or less extensive local lesions are produced, depending on the virulence of the culture, the seat of inoculation and the susceptibility of the animal. In rabbits lobar pneumonia has been produced by inoculation into the pleura, trachea, blood stream or subcutaneous tissue.

Occurrence in the Body

The pneumococcus is present in the nose, mouth and pharynx of a large percentage of individuals. It is encountered more frequently in crowded cities than in country districts. It persists for weeks and months in the mouths of convalescents from pneumonia, and it reaches the mouths of those

who are in the vicinity of pneumonics. It is found frequently in the conjunctiva and occasionally in the deeper air passages. That it may reach the stomach and intestines with the sputum is apparent, and it has been found there as the cause of diphtheritic enteritis, a condition which may be followed by pneumococcus peritonitis or general infection.

Entrance into the Lungs.

The lungs are infected by inhalation of the cocci. Suspended in droplets of saliva or mucus, or adherent to foreign particles, they may be carried fairly deeply into the bronchial tubes. That they ever reach the alveoli by this means alone is questioned by many. Two factors would seem to prevent their being carried to the alveoli by currents of inspired air: First, foreign bodies or infected droplets are likely to strike and adhere to the walls of the respiratory passages before they have traversed a great length, and from this situation may again be carried out by the action of the ciliated epithelium or coughing; the tortuous passages of the nose and its hairs and moist surfaces arrest many micro-organisms. Second, the velocity of the inspired air is greatly reduced or is nil by the time the particles might have reached the alveoli, a condition which renders their arrest all the more probable. Nevertheless, pneumococci do reach the alveoli, and by some it is supposed that even in health they are carried there more or less constantly and are as constantly destroyed. Occasionally they have been found in the parenchymatous tissue of the lungs of individuals who have died of other than pneumococcal infections or of non-infectious diseases. In order to show that micro-organisms may be carried into the paren-

chyma by inspiration Nenninger allowed animals to inhale a spray containing *Micrococcus prodigiosus,* and killing the animals after one-half hour, was able to cultivate the coccus from the base of the lungs where only alveoli and the finest bronchial branches were present (cited by Weichselbaum).

Lymphogenous and Hematogenous Infection.

Various other agencies have been suggested by which the cocci may be carried to the parenchymatous tissue. For example, during the forced respiratory efforts which accompany coughing they may be carried from the bronchial branches into the alveoli. Or the organisms having reached the bronchi, may be carried through the walls of the latter, perhaps by the leucocytes, and reach the alveoli directly through the lymph channels or after having caused infection in the peribronchial lymph glands. Others express the opinion that pneumonia follows blood infection in many or most instances, i. e., that the infection is hematogenous, the cocci having reached the blood in some obscure manner. That the infection may be hematogenous is shown by the occasional occurrence of pneumonia secondary to pneumococcus infection in other parts of the body.

Conditions for Infection.

Knowing the fairly constant presence of pneumococci in the upper respiratory passages in the normal individual, it seems certain that some unusual condition must arise to precipitate infection of the pulmonary tissue. Concerning the nature of these conditions, we have little but theories. They may rest either with the microbe or the individual, or with both. The pneumococci which are normally on the mucous surfaces may undergo an increase in virulence, or more virulent organ-

isms from the outer world, or from pneumonic patients, may be inhaled. The latter condition is an important one in relation to the contagiousness of pneumonia and the development of epidemics. Park and Williams found a larger percentage of virulent organisms in the sputum of pneumonics than in that of normal persons. It is possible that the pneumococcus in being passed from one patient to another undergoes an increase in virulence, similar to the increase which may be obtained by passing bacteria through animals.

Decrease of Resistance.

On the other hand, it is very probable that essential changes take place in the individual, changes which in some may cause the lowered resistance which is so often referred to as a condition for infection. Exposure to cold has long been known as an important predisposing factor, although we continue in ignorance of its precise effects. Animals are more susceptible to pneumococcus infection after artificial reduction of the body temperature. It is possible that a lowered body temperature may decrease antibacterial activities; that the activity of the bactericidal ferments of the plasma or of the leucocytes may be suppressed, or phagocytosis may be inhibited so that organisms which reach the bronchi and peribronchial lymphatic structures are allowed to proliferate. It is probable that in health the leucocytes continuously pass through the bronchial and alveolar walls where they may englobe foreign particles (coal dust) or bacteria, and leucocytes are present on the mucous membranes of the mouth cavity. Following exposure and the reduction of the body temperature, or following the prolonged inspiration of cold air, the activity of the phago-

cytes may be inhibited so that cocci which reach these surfaces are not ingested and continue to proliferate, or the same conditions may decrease the exudation of the leucocytes from the vessels. It is possible also that the activity of the ciliated epithelium is reduced similarly so that the cocci are not so readily carried to the exterior.

Other Predisposing Factors.

Extreme exposure is not always followed by pneumonia, however, and not all cases of pneumonia are preceded by exposure; many other conditions may predispose to infection, as a lowered resistance due to alcoholism, other infections or to non-infectious processes. That certain local conditions may favor infection is indicated by the frequency with which individuals with chronic tuberculosis of the lungs die of pneumococcus pneumonia, and the development of the disease in areas of hypostatic congestion. Age is of influence. "To the sixth year the predisposition to pneumonia is marked; it diminishes to the fifteenth year, but then for each subsequent decade it increases" (Osler). The cause of these variations is not known, although the rise in later years may be associated with increased exposure.

The conditions which predispose to infection are now the subject of active study in many laboratories, and the commission which the New York Department of Health has established for the study of acute respiratory diseases has already made important observations as to the prevalence and virulence of pneumococci.

Complications.

Many observers have found pneumococci in the blood in a large percentage of the cases, and recent work by Rosenow indicates that the blood is probably infected in all cases at some stage of the dis-

ease. This being the case, the frequency with which pneumococcus infections occur in other organs as complications of pneumonia is readily understood. Pleuritis is present almost constantly, pericarditis frequently, and the peritoneal cavity is invaded not infrequently by way of the diaphragm, with general peritonitis as the occasional result. In pneumococcus pleuritis the exudate is frequently of a serous character. Endocarditis, meningitis and arthritis are frequent complications. Conjunctivitis, otitis media, cutaneous or subcutaneous infections, intramuscular abscesses and osteomyelitis may develop. The kidneys and liver usually show acute degenerations.

Diplococcus pneumonia occurs as a complication in typhoid, diphtheria, tuberculosis, influenza, erysipelas and other infections, the organism of the primary infection also being found in the lungs. Not infrequently staphylococci,, streptococci, *Micrococcus catarrhalis,* or the bacillus of Friedlander, are found with the pneumococcus, the latter being the predominating organism. Recent work from Phipps' Institute (Flick, Ravenell and Erwin) suggests that the pneumococcus may be an exciting cause of pulmonary hemorrhage in the tuberculous.

Prophylaxis. Prophylactic measures are largely of an individual character. One should not come in contact unnecessarily with those suffering from pneumonia. The susceptible should be guarded against exposure; pneumonia should be considered as a contagious disease, the cases isolated as such, the sputum disinfected, and rooms cleaned with moist antiseptics rather than by dusting and sweeping; the sick room should be flooded with sunlight, and the

mouths of convalescents disinfected. Expectoration in public places should be limited. To what extent the dust-laden atmosphere which prevails in most of our large cities is a factor in causing pneumonia is unknown. Vaccination is not yet an established procedure.

Immunity and Susceptibility.

It is probable that the susceptibility of man varies greatly. Under equal conditions of exposure not all contract pneumonia, and an individual who eventually contracts the disease may have undergone many similar exposures previously. Klemperer introduced a culture of the pneumococcus which was virulent for rabbits under his skin without suffering more than temporary disturbance.

Recovery.

Recovery seems to indicate an acquired immunity or resistance which is by no means permanent, and often is of very short duration. One may have as many as eight or ten attacks of pneumonia, the intervals between attacks being from three to five years on the average (Griswolle). What the recovery or acquired resistance depends on is unknown. The marked leucocytosis of pneumonia, and the known phagocytic power of the leucocytes for the diplococcus, suggest strongly the importance of the leucocytes for recovery. The serums of convalescents and of immune animals show no increased bactericidal power for the organism, nor are they strikingly antitoxic.

Serum Properties and Opsonins

Beginning with Fraenkel (1886), many have shown the possibility of increasing the resistance of susceptible animals to the pneumococcus by injecting first dead or avirulent and then virulent cultures; in this way the subjects can be made to withstand many multiples of the minimum fatal

dose. Culture filtrates and precipitates (the pneumotoxin of F. and G. Klemperer) have been used for similar purposes. The serum of immune animals, and in some instances of convalescents, has been found to have a protective effect when injected into other animals, and by some a curative effect is claimed when the serum is given shortly after infection. Mennes made the interesting observation that "normal leucocytes only become phagocytic toward pneumococci when they are lying in the serum of an animal immunized against this bacterium" (Muir and Ritchie). This action may have been due to the effect of the opsonins which Wright and Douglass have shown to be essential for the phagocytosis of pneumococci. According to Neufeld and Rimpau, antipneumococcus serum is not bactericidal, but through the influence of bacteriotropic substances (opsonins ?) which it contains renders the cocci more susceptible to phagocytosis.[1] Likewise, Park and Williams found antipneumococcus serum from the sheep to be protective for mice and to stimulate phagocytosis. The correspondence between bacteriotropic action and protective power was variable, however, so that it did not appear certain that the protective power of the serum was due entirely to its influence on phagocytosis. We are, of course, not sure that events in the animal body correspond with those in the test-glass.

Phagocytosis.

Serum Therapy and Agglutination.

Some of the serums which have been prepared have been used therapeutically in man. The results have not been sufficiently satisfactory to put

1. This bacteriotropic substance, according to Neufeld, differs from the opsonin of Wright in that it is not destroyed by low degrees of heat.

them on a good basis, although some favorable reports have been given.

Serum of Roemer.

The serum of Roemer, which is best known at the present time, is obtained by immunizing different kinds of animals with several strains of pneumococci. The receptor apparatus of different strains probably differ; hence, a serum obtained by immunization with several strains probably would be effective against a large variety of pneumococci. Furthermore, since different animals may respond to immunization with a given organism by the formation of amboceptors with different complementophilous haptophores, a theoretical advantage is to be gained by mixing immune serums from several animals. The amboceptors of one or more of the serums may be susceptible to activation by the complement of the patient's body, whereas if only one serum were used the chance of such activation would be decreased. Pässler, in summing up the results obtained in the treatment of 24 cases with this serum, finds the course of the disease shortened, the temperature reduced and a tendency to limit the extension of the disease to other parts of the lungs.

Agglutination.

The serum of pneumonic patients shows an increased agglutinating power for the pneumococcus. The maximum is reached at or near the time of crisis, but rarely has a higher value than 1 to 50 to 1 to 60 (Neufeld, Rosenow). It disappears quickly after recovery. In immunized animals the agglutinating power may be pushed to much higher limits. Not all strains yield agglutinins equally, and not all are agglutinated equally by the same serum. According to Collins, pneumococci fall into different groups, depending on their agglu-

tinable properties; the same author determined the presence of group agglutinins in an immune serum. Neufeld states that avirulent strains were not agglutinated by the serum of pneumonic patients.

OTHER INFECTIONS BY THE PNEUMOCOCCUS.

Complicating infections by the pneumococcus during the course of pneumonia were mentioned above. They may occur by way of the lymph channels, as in pleuritis, pericarditis and peritonitis (through the diaphragm), by continuous extension, as in infection of the bronchi, nose and, perhaps, the middle ear, or as metastatic infections following the invasion of the blood stream by the organisms. It is undoubtedly in the last named manner that meningitis, endocarditis, arthritis, muscular and subcutaneous abscesses arise.

Mode of Infection.

Other infections by the pneumococcus occur independent of the existence of pneumonia. Such conditions are alveolar abscesses, conjunctivitis, dachryocystitis, serpent ulcer of the cornea, inflammation of the middle ear, meningitis, enteritis, rarely peritonitis, and pneumococcus septicemia which may be complicated by infection in various organs. The eye is exposed to infection from without and the ear from the pharynx. Primary pneumococcus meningitis occurs both sporadically and epidemically, although the meningococcus is a more frequent cause. The organisms may gain entrance through the middle ear or nose, or through the circulation from a primary focus in another organ, perhaps an undiscovered focus. Preceding and during meningitis the nose is not infrequently the seat of pneumococcus rhinitis, and

the organisms may be carried from the nose to the meninges by way of the lymph channels. The blood may be infected secondarily. Pneumococcus meningitis is almost invariably fatal. The organism causes chronic meningitis less frequently than the meningococcus. Infection of the peritoneum may follow an intestinal infection; a pure pneumococcus infection of the peritoneum in the absence of pneumonia is extremely rare. Pneumococcus infections of the eye, ear, intestines and peritoneum are likely to be accompanied by other organisms.

Pneumococcus conjunctivitis occurs in epidemic form and the same precautions should be taken to limit it as for the limitation of influenza conjunctivitis.

Serpent ulcer of the eye, a progressive phagedenic process in the cornea, has the pneumococcus as its essential cause, although other organisms may be present. Roemer treats the condition with an antipneumococcus serum and claims that he is able to arrest the process if the treatment is begun sufficiently early. The serum is injected beneath the conjunctiva.

II. STREPTOCOCCI.

Discovery of Pyogenic Cocci.

When wound infections, cases of septicemia and pyemia were first studied bacteriologically, various names were applied to certain cocci which were found. Such were the *Microsporon septicum* of Klebs and the *Coccobacteria septica* of Billroth and others. Pasteur recognized such organisms and cultivated them at an early date, but Ogsten, in 1880 to 1884, using the newly-devised technic of Koch, was the first to recognize two sorts of

pyogenic cocci, to which he gave the names of streptococci and staphylococci. The former grew in the form of chains and the latter in clusters. In 1883 Fehleisen obtained the streptococcus in pure cultures from cases of erysipelas. Rosenbach determined more exactly the significance of streptococci in wound infections and septicemia, and gave to the organism the name of *Streptococcus pyogenes.*

Morphology. The typical streptococcus is a spherical or spheroidal cell, about one micron in diameter, which grows in the form of chains of varying length. Division takes place in one direction only. Variations in form, such as diplococcus-like cells in pairs or chains, or elongated cells resembling bacilli, represent accidental stages or anomalies in division. Streptococci commonly appear as diplococci in the blood and tissues of the infected. Unusually large cells may be involution forms. The difficulty of distinguishing the pneumococcus from the streptococcus has been mentioned. At one time it was thought that streptococci could be separated into those which grew in long chains (*S. longus*) and those which produce short chains (*S. brevis*). Although these names are still used for convenience, they are not well grounded, since the length of the chains is not an inherent property; one form may be changed into the other by appropriate methods of cultivation. Similarly the *S. erysipelatis* of Fehleisen is not a specific organism for erysipelas, since strains from other sources are able to cause experimental erysipelas in man. Streptococci growing in short chains may be cultivated from the normal mouth cavity and they are usually of low virulence for animals. On the other hand, *S. longus* is more often obtained

from wound infections, septicemia and malignant tonsillitis. Capsulated strains of high virulence are occasionally found in the body. Ordinarily, however, streptococci are not surrounded by a capsule. The *Streptococcus mucosus capsulatus* may be a pneumococcus. Although streptococci are described which do not stain by Gram's method, those with which we are concerned invariably react positively. Streptococci are never motile, possess no flagellæ and form no spores.

Cultivation.

Streptococci grow better in a neutral or slightly alkaline medium than in one of acid reaction, but virulence is lost rapidly. They may be cultivated indefinitely in media which contain serum or ascitic fluid, but even here virulence disappears gradually; frequent transplantation is necessary. In bouillon those strains which produce short chains or grow as diplococci cause a diffuse clouding of the medium, whereas those growing in long chains sink to the bottom, leaving a clear overlying fluid. Streptococci demand little oxygen, all are facultative anaërobes and some are said to be obligate anaërobes; obligate anaërobes may be cultivated from the vagina and intestines. The optimum temperature for growth is 37° C.

Resistance.

When dried, streptococci live for from ten days to several weeks; they are destroyed more quickly in the presence of sunlight. Susceptibility to antiseptics depends on the nature of the medium in which they are suspended or imbedded. When unprotected by bouillon or other fluid they are killed in a few seconds by 1/1000 corrosive sublimate and 3 per cent. carbolic acid (Fehleisen); when in bouillon, by 1/1500 corrosive sublimate and by 1/200 carbolic acid in fifteen minutes. Ly-

ing on a mucous surface, where they are imbedded in mucus or tissue fluids, they are protected against antiseptics to some extent. They are fairly resistant to heat, being destroyed by a temperature of 70° to 75° C. in one hour (v. Lingelsheim).

Virulence. Streptococci vary widely in their pathogenicity. Cultures which are entirely non-pathogenic for animals are frequently cultivated from nature and from man. As a rule, however, the long chains obtained from pathological processes in man are pathogenic for rabbits and mice. Their virulence is very labile, and by passage through suitable animals (rabbit, mouse) it may be pushed to a very high point; in doing this, however, the original virulence of the culture undergoes modifications. For example, Marmorek so increased the virulence of one strain that the milliardth part of a c.c. was fatal for rabbits, but it had lost its pathogenicity for man, as shown by inoculations into carcinomatous patients. Hence the pathogenicity of cultures for animals is not a good index of their virulence for man. Those which produce long chains in bouillon are more pathogenic than those forming short chains (v. Lingelsheim).

Rabbits and mice are the most susceptible animals. The rat, guinea-pig and cat, and larger animals, as the horse, goat and sheep, are less susceptible. A bouillon culture of which .01 to 1.0 c.c. will kill a mouse or rabbit in from one to four days is considered of high to moderate virulence. Virulent cultures cause systemic infection, regardless of the method of inoculation. Less virulent cultures produce changes which are more localized in character and which may heal: abscesses, areas of necrosis and erysipelatous inflammations.

Endotoxin.

The properties on which the virulence of streptococci depends are little understood. The conflict of opinion concerning many points probably depends on the use of different strains of the organism in experimental work. The amount of endotoxin which virulent strains contain is subject to great variations. Aronson found practically none in the killed cells of a very virulent strain. It seems probable that the endotoxin is rather susceptible to heat, since cultures which are killed by mild methods, as by chloroform, are more toxic than those which are killed by heat. The filtrates of old bouillon cultures are more or less toxic. A strong "toxin" was prepared by Marmorek by growing a virulent strain in a mixture of serum and bouillon for three months and filtering the culture. More recently he uses a medium containing glycocol and leucin. Toxic precipitates from fluid cultures have also been obtained. Bouillon filtrates of virulent cultures after two to fourteen days of growth have low toxicity (Aronson).

Streptocolysin and Leucocytic Toxin.

Besredka, and later G. F. Ruediger, showed that virulent streptococci produce a hemolytic toxin when grown in various heated serums. Ruediger proved that this hemolysin (streptocolysin) is a true toxin, possessing a haptophorous and toxophorous structure. This discovery has an important bearing on the fact that the blood in fatal streptococcus infections, especially in rabbits, is often more or less laked. Streptocolysin is destroyed by a temperature of 70° C. in two hours, by peptic digestion, deteriorates rapidly at ordinary temperatures, and is non-dialysable. Certain normal serums contain antistreptocolysin (Ruediger). Another significant discovery by Rue-

diger is that virulent strains, when grown in serum and ascitic fluid, produce a substance which kills leucocytes and inhibits phagocytosis. This may explain the failure of leucocytes to take up virulent organisms, whereas non-virulent strains are readily phagocytized. Lingelsheim states that strains cultivated from subacute or chronic processes produce more soluble toxin (nature unknown) than highly virulent strains. Not all toxic filtrates contain streptocolysin, the hemolysin being independent of other toxic constituents (Simon). Lingelsheim concludes that the infectiousness of streptococci is not explained by the toxic properties which have been demonstrated. He lays stress on their resistance to the bactericidal activities of the tissues and tissue fluids. It is safe to say that up to the present time the essential toxin of the streptococcus has not been demonstrated.

Pathologic Processes.

Streptococci are the frequent cause of wound infections, the most common cause of lymphangitis and diffuse inflammations of the subcutaneous and intermuscular connective tissues (cellulitis), endometritis and puerperal septicemia, endocarditis and tonsillitis, are often the exciting organisms in pneumonia (lobular, usually), bronchitis, meningitis, inflammations of the serous surfaces (pericardium, pleura, peritoneum joints), enteritis and suppurative processes in the middle ear. They are the exclusive cause of erysipelas, which occurs naturally, and serious attempts have been made to show that they are etiologic factors in scarlet fever and rheumatic fever. The streptococcus is the most common organism found in the lesions of impetigo contagiosa, although it may be mixed with other bacteria, especially the staphylococcus.

Occurring as mixed infections in pneumonia, tuberculosis, scarlet fever, enteritis and other processes, they cause grave and often fatal complications.

Erysipelas.

Not all streptococci are able to cause erysipelas, and a streptococcus cultivated from a case of erysipelas is not able to cause the disease in all individuals. Furthermore, cultures obtained from other sources (phlegmon) may produce the disease. (Koch and Petruschky.) Koch produced an erysipelatous inflammation with a staphylococcus. It has been suggested that streptococci which cause erysipelas, rather than some other process, do so because of some peculiarity in their virulence or in the resistance of the individual, or perhaps both. Another suggestion is that this type of infection depends on some peculiarity in the skin and subcutaneous tissue of the susceptible. The conditions are obscure. The infection atrium is not always known. In facial erysipelas entrance probably is gained through the mucous membrane of the nose in many instances. Erysipelas is a wound infection in most or all instances, although the atrium often escapes observation. The cocci lie principally in the lymph spaces and interspaces of the connective tissue. They are rarely to be cultivated from the scales or the fluid of blisters, but may be obtained from skin which is excised from the border of the inflamed area. (Fehleisen.) They probably are not excreted through the unbroken skin.

Lymphangitis.

Erysipelas is an inflammation of the superficial lymphatics of the skin, while in lymphangitis the deeper lymphatics are involved. Thrombosis of the lymphatic vessels, congestion of the adjacent

blood vessels, causing reddened streaks and local hemolysis (?), are distinguishing local features. Metastases occur to adjacent lymph glands and the infection may become general. In this process, as well as in wound infections, thrombosis of the adjacent vessels may occur, which may be the first step in the production of pyemia with multiple points of infection. Cellulitis may also be caused by the staphylococcus alone or infection with the latter may be superimposed on a primary streptococcus cellulitis.

Pneumonia. Pneumonia produced by the streptococcus may either be primary or secondary to infection in other parts of the body. Characteristically, it resembles the lobular type in the occurrence of multiple foci, which present a smooth surface on section and are very rich in cells. It occurs less frequently as the cause of lobar consolidation, and very frequently as a mixed infection in pneumonias caused by the pneumococcus and other organisms. Streptococcus infection of the lungs in pulmonary tuberculosis is a serious and frequent complication of the latter disease. It produces a septic condition, involves adjacent healthy tissue, and its rôle in causing consolidation and liquefaction of the tissues predisposes of hemorrhages. In cultures the streptococcus is said to inhibit the growth of the tubercle bacillus, and it has occasionally been noted that the tuberculous, after suffering a streptococcus infection (erysipelas), show an improved condition!

Meningitis. Primary streptococcus meningitis is rare or of doubtful occurrence. It frequently is secondary to otitis media, and has been noted following ton-

sillitis, facial erysipelas, pneumonia, endocarditis and as part of a pyemic process.

Enteritis.

Streptococci are perhaps the most important cause of enteritis in children, the inflammation often being membranous and accompanied by desquamation of the epithelium and by hemorrhages. It is not infrequently followed by peritonitis and septicemia. Virulent organisms probably reach the intestines through milk in many instances. Escherich found streptococci in nearly every sample of milk which he examined. Digestive disturbances due to other causes predispose to infection. The organisms are nearly always present in the intestines of the adult, but cause enteritis less frequently than in children.

Vagina and Uterus.

The normal vagina does not offer a good culture medium for pathogenic bacteria, although streptococci are occasionally found there. They occur more frequently in those who have borne children. The vagina tends to purify itself mechanically and by the acid nature of its secretions. If the secretion for any reason becomes alkaline, as in catarrhal conditions, or if it contains blood and serum, which provide a good culture medium, virulent streptococci proliferate. Infection takes place through denuded surfaces and tears; endometritis, metritis, parametritis, salpingitis, peritonitis and sepsis may follow. Thrombosis of the blood vessels may be followed by the development of pneumonic foci.

Upper Respiratory Passages.

Streptococci are probably always present on the tonsils, the mucous membrane of the mouth, very frequently in the sputum and not infrequently on the mucous membrane of the anterior nares. Presumably they proliferate under inflammatory con-

ditions from whatever cause, finding in the serum and plasma which exude a medium favorable for growth and the development of virulence. They are of great significance in severe local inflammations, as in diphtheria and scarlatina, and when general resistance is lowered, as in typhoid, typhus, variola, measles, etc. Lingelsheim characterizes their relation to diphtheria as follows: they injure the tissues locally, penetrate beneath the membrane into the tissues and take part in the formation of the membrane; they increase the virulence of the diphtheria bacillus; alone, or in conjunction with the diphtheria bacillus, they may invade the lungs, causing bronchopneumonia, or enter the circulation and injure various organs, but particularly the kidneys. Their method of entering the lungs from the upper respiratory passages probably is similar to that involved in pneumococcus infection. Furthermore, having obtained a footing in the pharynx, for example, they may reach the bronchi and perhaps the alveoli by extension along the surface.

Streptococci are usually the essential organisms in follicular tonsillitis, are frequently found in alveolar abscesses, but in both instances may be mixed with other organisms, especially the staphylococcus and pneumococcus. Streptococci in the throat may appear in diplococcus form in fresh preparations. Beginning primarily in the nose, tonsils or pharynx, streptococcus infection may extend to the adjacent sinuses, the middle ear, meninges, or through the tonsils may cause systemic infection with endocarditis as a frequent complication.

The endocarditis caused by streptococci usually

Endocarditis.

is vegetative in character, but may be ulcerative, and may result in metastatic foci of infection (e. g., septic infarcts). Infarcts from streptococcus endocarditis are not always infected, however. Not infrequently the vegetations contain staphylococci as well as streptococci.

Rheumatic Fever.

Since 1867, when Salisbury described a fungus which he called *Zymotosis translucens*, many microorganisms have been described and cultivated from the joints, blood, endocarditic and pericarditic lesions and from the tonsils in acute articular rheumatism. Among them were the "Monadinen" of Klebs (1875), short bacilli by Wilson (1885) and others, staphylococci and streptococci by Weichselbaum (1885) and by many others, and an anaërobic bacillus resembling that of anthrax by Achalme (1890). Streptococci have been found more frequently than other organisms. The bacillus of Achalme acquired considerable prominence at one time, being found in rheumatism in a number of cases, but it has been found since in other conditions, and normally, and Achalme himself gave up his original claims for its etiologic significance. The organism, possibly, is identical with *B. aerogenes capsulatus* of Welch (Harris). Many of the observations are of little value, since the cultures were made postmortem, when contaminations and agonal invasions by other organisms could not be excluded. The conditions were very confusing, however, since the injection of pure cultures occasionally produced arthritis, pericarditis and endocarditis in animals. This was the case with a short anaërobic bacillus or diplobacillus cultivated by Thiroloix, and by Triboulet, Coyon and Zadoc (1897).

In 1897-98 Triboulet and Coyon cultivated from the blood of five cases of rheumatic fever a diplococcus, pure cultures of which caused arthritis, endocarditis, etc., in rabbits. Similar observations have been made by Westphal, Wassermann and Malkoff, Poynton and Paine, Beaton and Walker and others, and the possibility of producing lesions characteristic of rheumatic fever by the inoculation of pure cultures into rabbits has been well established. Although the organism was called a diplococcus by the discoverers, it can not be distinguished from the ordinary streptococcus pyogenes by cultural tests. These discoveries do not, however, put this particular streptococcus on a satisfactory basis as the cause of the disease, since streptococci from various sources are able to cause experimental arthritis in rabbits (Cole, Harris). It seems that virulent streptococci from whatever source have a predilection for serous surfaces. This is apparent from the frequency with which the joints, endocardium, etc., are involved in streptococcus septicemia in man. The view of Singer and of Menzer that "acute rheumatism is simply one of the many manifestations of streptococcus invasion" (Harris), finds some justification in the streptococcus tonsillitis with which the disease usually begins, the recovery of streptococci from the lesions and the production of these lesions in rabbits by the injection of pure cultures. The fact remains, however, that streptococci can not always be cultivated from the lesions of rheumatic fever; hence it is possible that the organism may exist as a mixed infection with more or less constancy, and that the real cause is as yet unknown (Phillip).

Relation of Streptococci to Scarlet Fever.

The theory that scarlet fever is of streptococcus etiology has been held particularly by Babes, Klein, Moser, Gordan and Baginsky and Sommerfeld. Some have held that streptococci isolated from the disease show distinctive properties and deserve the name of *Streptococcus scarlatinæ*. This, however, is not agreed to by most bacteriologists, the organisms not differing from streptococci obtained from various sources. The organisms are not found constantly in the erythematous eruption.

Virulent streptococci are found on the tonsils almost invariably in scarlet fever. In 65 per cent. of the cases a membrane is formed (Ranke), and this is often due to the streptococcus, which is sometimes, however, associated with diphtheritic infection. The frequency with which streptococci invade the blood during scarlet fever is related to the severity of the disease. Occasionally they are found in mild cases, which run a short, uncomplicated course, but "more frequently in severe and protracted cases, in which there also may develop local complications and clinical signs of general infection, such as joint inflammations" (Hektoen). Baginsky and Sommerfeld found streptococci in the blood and organs of each of eighty-two fatal cases. Hektoen states, however, that streptococcemia is not necessarily present in fatal cases.

At the present time there is not sufficient ground for considering streptococci as the specific agent in scarlet fever, although they are undoubtedly the cause of the most frequent and serious complications. The mortality of the disease probably is greatly raised by mixed infections with the streptococcus.

Streptococcus filtrates or cultures may cause degenerative changes in the spinal cord (Homén and Laitinen).

Beneficial Influences.

Certain strains of streptococci are said to exercise a curative effect in experimental anthrax. Emmerich and di Mattei found that by intravenous injection of the cocci rabbits could be saved from an anthrax infection which otherwise would prove fatal in forty-eight hours. This result can not always be obtained, and it may be that only certain strains have this effect (Zagari, cited by Lingelsheim). It is noted occasionally that lupus improves or actually heals following an attack of erysipelas. A reputed effect of a similar nature in tuberculosis of the lungs was mentioned above.

Effect on Sarcoma.

The rather old observation that an attack of erysipelas often causes a decrease in the size of malignant tumors, especially sarcomas, received some confirmation from the experimental work of Fehleisen. With the hope of reproducing erysipelas with pure cultures, Fehleisen had inoculated streptococci into those suffering from such tumors. Among six patients so inoculated, a decrease in the size of the tumor was noted in five. Killed cultures were tried without effect. Coley's mixture of killed cultures of the streptococcus and *Bacillus prodigiosus* received rather extensive trial as a substitute for living cultures of the streptococcus, and in many instances improvement and even cures have been reported. Others have had no favorable results. Senn used the preparation in twelve cases of inoperable sarcoma "with negative results." The *Bacillus prodigiosus* is supposed in some way to increase the efficacy of the strepto-

coccus toxin; it contains a toxic protein. These toxins seem to have no influence on carcinomas.

Immunity and Susceptibility.

Concerning the natural susceptibility and immunity of man to infections with the streptococcus little is known. It seems probable that the unimpaired mucous surface resists invasion by the organisms which occur constantly in the mouth cavity; the physical protection of the intact surface, the rapid desquamation of epithelium, the rapid excretion with the saliva, the inhibiting influence of the saliva on the proliferation of bacteria and the destruction of bacteria by the leucocytes which constantly appear on the mucous surface are probably important factors in this local resistance. Congestion of these surfaces, especially the tonsils, from any cause, as from exposure, or the occurrence of some other infection, as may be the case in scarlet fever, may lower the local protective powers. And, as stated, the serum and plasma which exude in simple (?) catarrhal conditions or other inflammations, provide a medium which favors the growth and development of virulence by streptococci.

Concerning the conditions which, in the body, antagonize infection, we are largely in the dark. It has been impossible to demonstrate antitoxic and bactericidal substances in the normal serum of man. Streptococci grow freely in fresh normal serum which contains no leucocytes. (Weaver and G. F. Ruediger.) Phagocytosis of streptococci first came under the observation of Metchnikoff, who in 1887 noted it as a striking occurrence in erysipelas. Only the microphages took up the cocci. The marked lucocytosis which is noted clinically suggests, but of course does not prove, that the

leucocytes take an active part in the destruction of the cocci. Experimental work showing such a relationship is not lacking, however. Bordet concluded that all the protection which guinea-pigs and rabbits show against streptococci is due to the phagocytes. In actual infection streptococci have often been found within the leucocytes of the blood and inflammatory exudates. (G. F. Ruediger.) Non-virulent or weakly virulent strains are phagocytized more readily than the virulent in experimental work. Ruediger also demonstrated conclusively that the streptococci taken up by polymorphonuclear leucocytes may be killed by the latter. Hence the evidence in favor of a protective rôle by the leucocytes is more than presumptive. Ruediger suggests the importance of the leucocytic toxin of the streptococcus for the development of infection. It may either kill the leucocytes or cause negative chemotaxis, and under these conditions proliferation of the cocci may proceed.

Acquired Immunity.

The streptococcus usually is classed with those organisms, infection with which does not cause the development of lasting immunity. A certain amount of immunity probably is established, however. This is suggested by the results of Fehleisen, who could not always cause second attacks of erysipelas by the inoculation of pure cultures into the susceptible. It is also suggested by the ease with which relatively high resistance can be produced in animals by brief immunization. A streptococcus infection of the horse which occurs naturally ("Druse") is said to produce immunity which lasts for a year or two.

One may immunize animals either with toxic filtrates or with killed and living cultures. The

filtrates are much less effective in producing immunity than the bacterial cells, and in the hands of many no immunity whatever could be established. **Immunization of Animals.**

A number of different principles have been followed in immunizing with cultures. It seems that virulent strains cause a higher degree of immunity and a serum of higher protective power for other animals than strains of low virulence. On this account Marmorek, and also Aronson, immunize horses with streptococci, the virulence of which has been pushed to a very high point by passing them through rabbits. Strong resistance is induced by this method, and the immune serum, particularly that of Aronson, shows distinct protective power for other animals. Such serums, however, have the highest protective power against the particular strain which was used for immunization, although the serum of Aronson is not devoid of protective powers against other pathogenic strains. Concerning the serum of Marmorek there are divergent opinions. In the hands of Marmorek it is highly protective in animal experiments; others have found it without value. The method of Marmorek and of Aronson rests not only on the basis that strains of the highest virulence will give the strongest serums, but also on the assumption of the unity of all pathogenic streptococci. If all are alike in their biologic and pathogenic properties, a serum which protects against one should protect against all. As pointed out, there is at present not sufficient ground for considering the streptococci of erysipelas, scarlet fever, rheumatism, sepsis, etc., as independent species. By cultivation and passage it is possible to so modify any **Unity or Multiplicity of Streptococci.**

one of them that it is indistinguishable from the others, on the basis of morphology and pathogenicity. On the other hand they are not all identical in some very important properties. For example, not all strains produce hemolysin to the same degree, and they differ greatly in their susceptibility to the action of an agglutinating serum. We have also to remember that pathogenicity for animals is not a reliable index of pathogenicity for man. From these confusing conditions we can only regard the question of unity or multiplicity of streptococci as an open one, which may be decided by future investigations.

Univalent and Polyvalent Serums.

The serums of Marmorek and Aronson are univalent serums, a single strain being used for immunization. Certain investigators, believing in the multiplicity of streptococci, utilize several strains in immunization. The serum of Denys is obtained by immunizing with several strains the virulence of which has been artificially increased. Such a serum would, theoretically, have a wider range of action than a univalent serum; it is polyvalent. Having in mind the fact that passing a culture through rabbits increases the virulence of the organism for the rabbit, but alters its virulence for the original host (man), Tavel, Moser and Menzer prepare serums on a different basis. Tavel employs several strains of streptococci cultivated from pathological processes in man, avoiding such alterations in virulence as would be caused by passing the cultures through animals. On the assumption that scarlet fever is a streptococcus disease, Moser immunizes horses with strains (about twenty) which are cultivated from cases of scarlet fever. In a similar manner, Menzer,

supposing that rheumatic fever is a streptococcus infection, immunizes with a number of strains cultivated from the tonsils of cases of rheumatism. Both Moser and Menzer avoid passage in order to retain the original biologic properties of the cultures.

Serum Protection.

In animal experiments, some of these serums, and particularly that of Aronson, have exhibited strong protective powers. Aronson's serum in doses of 0.0004 to 0.0005 c.c. protects a mouse against ten fatal doses of the streptococcus given twenty-four hours later than the serum. A serum of which 0.01 c.c. protects against a dose known to be fatal is considered of normal strength. The present serum, then, is of twenty- to twenty-five-fold value. In some instances animals can be saved when the serum is used some hours after infection, but this period is a brief one.

Serum Therapy.

Statements concerning the value of antistreptococcus serums in treating human infections are very conflicting. The serum of Marmorek has been given more general trial than any other, and the results have not been satisfactory. Favorable effects, such as the lowering of temperature and improvement in the general condition, have been reported, but the serum possesses no distinct curative power in established infections. Koch and Petruschky deny that it has a prophylactic power in experimental erysipelas. Escherich, by using the serum of Moser, and Baginsky, by using that of Aronson, observed a shortening of the course, a reduction of the fever and general improvement in cases of scarlet fever. Moser claims that it reduces the mortality of the disease. The use of antistreptococcus serum in the treatment of scarlet

Scarlet Fever.

fever does not commit one to the streptococcus etiology of the disease, but rather to the importance of streptococcus complications; hence, if the danger of these complications can be reduced by antistreptococcus serum its use is justified. It remains for future work to demonstrate to our satisfaction that it has such value.

Rheumatism.

What has been said concerning the treatment of scarlet fever with the serums of Moser and Aronson also applies to the treatment of rheumatism with the serum of Menzer. Favorable reports have appeared concerning its value, but a sufficient mass of experience has not accumulated to permit of satisfactory judgment. "So much appears from observations in man that the different streptococcus serums are harmless" (Dieudonné).

Properties of Serum.

As nearly as can be learned at present, antistreptococcus serum is protective (and curative (?)) because of its ability to stimulate phagocytosis, rather than because of serum antitoxins or bacteriolysins. This was indicated by the observations of Bordet in animal experiments, in which marked phagocytosis of streptococci took place in the peritoneal cavity of immunized animals, but very little in normal animals. A similar condition was noted in the test-glass experiments of Denys and van der Velde. A mixture of normal rabbit serum and leucocytes showed very little phagocytosis of streptococci, whereas the addition of antistreptococcus serum caused active phagocytosis, with death of the cocci. The presence of a definite substance in the serum which stimulated phagocytosis was conceived by van der Velde and also by Lingelsheim. It was heat-resistant (62° to 65° C.), and was not destroyed

Stimulation of Phagocytosis.

by dilute acids and alkalies (cited by Lingelsheim). Hence its resistance is greater than the opsonins of Wright and Douglass, but perhaps not greater than the bacteriotropic substances of Neufeld. It is probable that some of these substances are heat-resistant and others heat-susceptible.

Agglutination.

The agglutinability of streptococci from different sources, and even from the same source, varies a great deal. Also the normal serums of man and animals have a variable agglutinating power for different strains of streptococci. By immunization with a given strain the agglutinating power is increased, but not uniformly for all strains. Commonly the strain used for immunization is agglutinated more strongly than heterologous strains, the latter sometimes undergoing no agglutination whatever. These variations do not depend on discoverable differences in the cocci or the diseases which they produce. A given antistreptococcus serum does not agglutinate equally all streptococci from cases of scarlet fever (Weaver). Also streptococci vary greatly in their ability to stimulate to the formation of agglutinins. On the whole those which produce long chains are more susceptible to agglutination and yield stronger serums than those with short chains. (Aronson, Tavel, v. Lingelsheim.) By passage the agglutinating properties undergo rather complex changes. The organism then produces a stronger agglutinating serum and is agglutinated more readily by this serum than the same strain which had not been passed through animals. If passage is discontinued it reverts to its former condition.

The variations are such that the agglutination

reaction is of little or no value in differentiating different types of streptococci.

As to the clinical value of the test for the diagnosis of scarlet fever, the conclusions of Weaver may be cited:

1. Of streptococci cultivated from cases of scarlatina, some are agglutinated by almost all scarlatinal sera, but at dilutions varying from 1/60 to 1/4000; others are agglutinated by the same sera with less constancy and at lower dilutions, and many are not agglutinated at all.

2. Streptococci cultivated from cases of scarlatina are agglutinated by sera from cases of lobar pneumonia and erysipelas at about the same dilutions as by scarlatinal sera, and in the case of erysipelas even at higher dilutions.

3. The same appears to be true of typhoid fever serum, so far as limited tests indicate, and to almost the same extent of puerperal-fever serum.

4. The agglutination reaction between the streptococci cultivated from cases of scarlatina and the serum from cases of scarlet fever is in no way specific, and can not be of any value as a means of diagnosis.

By growing streptococci on a medium which contains serum (serum bouillon), they form fewer and shorter chains and are better suited for agglutination tests.

III. STAPHYLOCOCCI.

Staphylococci are spherical cells from 0.7 to 0.9 microns in diameter, typically, and by light staining are often seen to consist of two hemispheres, which are separated by a delicate cleft. In pus they are found in small groups of two to nine or

ten, ocasionally as diplococci, tetrads or very short chains.

Cultivation and Biologic Properties.

They are luxuriant growers on nearly all media which are suitable for bacteria, preferring, however, a slightly alkaline reaction. Growth is best in the presence of oxygen, but proliferation occurs in its absence. Sputum, serum and ascitic fluid are favorable media, and in the last two the cocci may be agglutinated. An alkaline reaction is produced in litmus milk, and the casein is precipitated and partly digested. The production of a proteolytic ferment is shown by liquefaction of gelatin and the formation of a clear zone about the colonies when grown in plates which contain coagulated serum (Loeb, cited by Neisser and Lipstein). Albumen is changed into peptone. Loeb distinguishes between a ferment which liquefies gelatin (gelatinase, a "collolytic" ferment), and one which digests albumen (tryptic ferment). Gelatinase is present in staphylococcus filtrates and normal serums are rich in antibodies for it. A fat-splitting ferment (lab ferment) is also present in the filtrates. The fact that the pus which is produced in staphylococcus infection does not coagulate may be due to the action of the proteolytic ferment, which digests the fibrinogen.

Ferments.

Staphylolysin.

Van der Velde had noted in 1894 that "staphylotoxin" (staphylococcus filtrates) cause hemolysis. Neisser and Wechsberg, in 1901, by growing the organisms in bouillon of suitable alkalinity, obtained hemolytic filtrates, giving the name of staphylolysin to the hemolytic principle. The hemolytic action of the staphylococcus is readily seen in cultures on blood-agar plates; a zone of hemolysis forms about the colonies. Erythrocytes of the

rabbit, when placed in bouillon cultures, undergo hemolysis. Staphylotoxin also produces hemolysis in the living body. The maximum production of staphylolysin occurs after a growth of nine to fourteen days in alkaline bouillon, and nearly all pathogenic strains yield it, whether aureus, albus or citreus. It is not formed by non-pathogenic strains. The toxin is destroyed by exposure to a temperature of 56° C. for twenty minutes. A specific antitoxin is present in many normal serums and may be increased by immunization with the toxin or the living organisms.

Leucocidin. In 1894 van der Velde found in the pleural exudates caused by inoculation with killed cultures of the staphylococcus a substance which is toxic for leucocytes, causing them to swell and the nuclei to disappear. This substance is called leucocidin. It is also produced in culture media, but the ability to form it is not so widely distributed as in the case of the hemolysin. Leucocidin is a true toxin, like the hemolysin; most normal serums contain antileucocidin, and the latter is increased by immunization with the toxin.* The suggestion is a natural one that leucocidin may be a factor in combating phagocytosis in infections with the staphylococcus. Neisser and Wechsberg devised a "bioscopic method" of determining the cytocidal action of the toxin. Living leucocytes, like other living cells, have the power of decolorizing methylene blue when oxygen is excluded. The destructive action of the toxin on the leucocytes is indicated by the failure of this reduction when the toxin is mixed with the cells.

* Leucocidin and staphylolysin will not yield antitoxins when their activity has been destroyed by heat.

Old culture filtrates (two to three weeks) show a rather high degree of toxicity for animals, producing extensive degeneration of the convoluted tubules in the kidney, a degeneration which is somewhat selective; hemorrhages into the intestinal mucosa; degeneration of the ganglionic cells, and fever. According to Levaditi, a mast-cell leucocytosis develops. The nature of the fever-producing substance is unknown. The toxicity of filtrates is said to be destroyed by a temperature of 56° C. **Toxic Filtrates.**

Cultures of the staphylococcus killed by heat show little toxicity, hence the question of the existence of an endotoxin is on no better basis than in relation to the streptococcus. It is possible that the heat required to kill the organisms destroys the endotoxin as well as the soluble toxins mentioned above. The virulence of the organisms has no direct relationship to the hemolysin or leucocidin, or the toxicity of the filtrates. Very pathogenic strains may produce a filtrate of little or no toxicity. It seems then that the essential pathogenic agent of the organism is unknown; as in the case of the streptococcus, its infectiousness may depend on its ability to resist the antibacterial activities of the body (phagocytosis, bacteriolysis (?)), which, of course, is a very indefinite assumption. What part the leucocidin plays in this resistance is not definitely known. **Endotoxin (?)**

The many varieties of the staphylococcus are differentiated on the basis of pathogenicity, pigment formation, liquefaction or non-liquefaction of gelatin, and other cultural properties. The *albus* differs from the *aureus* only in its inability to form pigment, and it can not be made to acquire this property. Pigment is formed most **Varieties of Staphylococcus.**

abundantly on potato, whereas little is formed on blood serum. Other pigment-forming varieties are: *S. cereus flavus, S. pyogenes citreus, S. scarlatinus* and *Micrococcus hematodes.* The *S. epidermidis albus* of Welch is of low virulence. Weichselbaum obtained a *S. endocarditis rugatus* from a case of endocarditis. Not all of these varieties produce soluble toxins. The pigment of *S. aureus* is an excretion product which is formed only in the presence of oxygen. It is insoluble in water, soluble in alcohol and ether, and gives the reaction of a lipochrome (i. e., the pigment may be saponified and gives the lipocyanin reaction in which the pigment turns blue when treated with concentrated sulphuric acid).

Resistance of Cocci.

Aside from wide individual variations, the resistance of staphylococci to heat depends on the concentration of the suspension, the nature of the medium (whether water, gelatin or pus), and whether the test is a dry or wet one (Neisser and Lipstein). Eighty degrees centigrade for one-half to one hour kills them under all conditions, and 60° C. for one-half hour kills many strains when suspended in bouillon. They are not killed by repeated freezing and thawing, and are very resistant to desiccation. When in the form of fine dust they die in twenty-eight days (Kirstein). Resistance to the action of sunlight is variable; some strains are killed in from three to five hours.

Staphylococci have fairly high resistance to antiseptics; when dried, corrosive sublimate (1/1000) kills them in two to three hours, and when imbedded in pus thirteen to sixteen hours are required (Ottaviano). Methyl alcohol, tincture of green soap and methyl violet are relatively good disinfectants. Methyl violet in a dilution of

1/10,000 kills them in five to fifteen minutes (Stilling). Formalin readily hinders development, but its bactericidal power is low. It is difficult or impossible to sterilize wounds infected with the staphylococcus by means of antiseptics.

Staphylococci are very widely distributed in nature and are to be found constantly in the superficial layers of the epidermis (*S. epidermidis albus*).

Leucotactic and Necrotizing Substances.

In infections the staphylococcus attracts large numbers of leucocytes, and the pus does not coagulate. The substance which attracts leucocytes is heat-resistant, since killed cultures will cause abscesses. In all but the most superficial lesions a characteristic result of infection is that of cell necrosis and the liquefaction of tissues. Neisser and Lipstein state that the necrotizing substance is a soluble toxin, since culture filtrates cause marked necrosis of the internal organs when injected (liver, heart, kidney). "Hence in staphylomycosis we can distinguish two active substances (von Lingelsheim), the leucotactic substance in the bodies of the cocci and the more important soluble staphylotoxin which exercises not only a local but also a general toxic action on the body" (Neisser and Lipstein).

Amyloid Degeneration.

Davidson produced amyloid degeneration in rabbits and mice by the injection of living cultures. This was confirmed by Lubarsch, who found the condition most readily produced in the chicken and with more difficulty in the mouse, rabbit and dog. It rarely results if suppuration is avoided. Killed cultures may be used.

Rabbits and mice are the most susceptible animals. The susceptibility of man is much greater.

Susceptibility of Animals. The organisms are most virulent for rabbits when injected intravenously, and a variety of lesions may result, as abscesses in various parts of the body (especially the kidney, heart and muscles), arthritis, endocarditis, etc. They are less pathogenic when injected into the pleural or peritoneal cavities. Rabbits are rarely to be infected by the feeding of cultures. In experimental infections degenerations of the axis cylinders in the white and gray matter, and of ganglionic cells, have been noted. The virulence of staphylococci is subject to great variations, and it may be increased by passage. In passing a culture through the rabbit eight times, Lingelsheim reduced the fatal dose for rabbits from 5 c.c. to 1/100 c.c., but a corresponding increase in virulence for the mouse and guinea-pig did not occur. Virulence for animals is not a reliable index of virulence for man.

Infections in Man. The staphylococcus is the most common pus producer in man. The most frequent infections are those of the skin, the organisms gaining entrance through the hair follicles rather than through the sweat ducts (Unna), resulting in such conditions as acne pustules, abscess of the skin and subcutaneous tissue, furuncles and carbuncles.

Skin. They are found almost constantly in the lesions of impetigo and often in pure culture. They have been much vaunted as a cause of eczema and they may be important as a secondary agent in this condition. The ordinary eczema probably is not parasitic in its cause, however (Sabouraud), and Neisser and Lipstein dispute the claim of Bender and others that eczema produced by staphylococcus filtrates is due to products of the microbe. This conclusion was justified, since the

same results were obtained with pure bouillon of similar alkalinity, the property could not be destroyed by heat, and antistaphylococcus serum was not able to prevent the dermatitis. Furuncles may be produced by rubbing virulent cultures into the skin, and abscesses by the injection of minute amounts. The staphylococcus causes purulent or seropurulent conjunctivitis rather infrequently. Primary infections of cavities which communicate with the surface, as the antrum of Highmore, the middle ear, nose, bronchi, lungs and tuberculous cavities, are not uncommon, and mixed infections with the staphylococcus in these localities is the rule, regardless of the primary cause. Infection of the mucous surfaces is less common than of the skin, however. It rarely causes aphthous inflammations, anginas, pneumonia, enteritis and cystitis when unmixed with other organisms.

Mucous Surfaces.

Staphylococcus septicemia of great virulence occasionally follows primary infection in other parts of the body, as wound infections, tonsillitis, puerperal infection (rare) and the so-called malignant carbuncles of the upper lip. In such instances a thrombophlebitis may be the means by which the organisms are poured into the circulation in large numbers. Inflammations of the serous surfaces, as the pleura, peritoneum and endocardium, are rarely primary, but follow systemic infection; the endocarditis usually is ulcerative and leads to metastatic foci of infection. Staphylococci have a particular affinity for the bony tissues, especially the bone marrow and the periosteum; they are the most common agent in the production of osteomyelitis and cause the so-called periostitis albuminosa. It is thought that they may persist in bone lesions

Septicemia.

Serous Surfaces and Bones.

for a period of years and later start up a fresh process. They involve the joints less frequently, but have been found, presumably as secondary agents, in acute rheumatism, and as the primary cause in pyemic abscesses of the joints. They are found occasionally in abscesses of the mammary and parotid glands, liver, lungs, and in pyorrhea alveolaris (rare). The cultivation of staphylococci in a pure state from the tissues does not of necessity indicate that they are the essential organism in the process (smallpox, rheumatism, etc.). Previous infections by many organisms, and likewise traumas, predispose to localization of the staphylococcus, and any infectious process in the skin is likely to be invaded by these organisms secondarily.

Mixed Infections.

Infections with the staphylococcus are characterized by both local and general leucocytosis, the local leucocytosis being a part of the suppurative process. As stated above, the staphylococcus contains a thermostabile constituent, which exerts a positive chemotatic effect on the leucocytes. Although it is possible to consider the accumulation of the leucocytes merely as the expression of this affinity, it has been shown with sufficient clearness that polymorphonuclear leucocytes are able to ingest living staphylococci and kill them.* They may be found within the leucocytes in both natural and experimental infections. When injected into the pleural or peritoneal cavity of the guinea-pig phagocytosis is well begun within one-half hour and reaches its height in four to five hours.

Leucocytes in Natural Immunity.

Experiments which were begun by van der Velde in 1894 demonstrate the bactericidal action of leu-

Bactericidal Action of Leucocytes and Leucocytic Exudates.

* Phagocytosis of staphylococci was first observed by Kirch in 1889.

cocytic exudates. The action is not so strong in the cell-free exudate as when the leucocytes are present, and when the leucocytes are caused to disintegrate by some means, as by alternate freezing and thawing, trituration, the action of leucocidin, or treatment with distilled water, the bactericidal power of the fluid is increased. Presumably the leucocytes discharge their bactericidal contents into the surrounding fluid as a result of such injuries. The nature of the bactericidal substance is not known exactly; from the fact, however, that leucocytes contain complement it has been suggested that they discharge this complement which then acts with amboceptors in the serum in destroying the organisms. It is possible that the cocci before they are taken up by the leucocytes have absorbed amboceptors and after their ingestion are susceptible to the action of the endocellular complement. In contrast to the distinct bactericidal power of the leucocytes stands the very low or entire absence of a similar action by both normal and immune serums. It would seem, then, that the most powerful agency in natural resistance to invasion by the staphylococcus is represented in the phagocytic and bactericidal activities of the leucocytes. Opsonins are essential for phagocytosis.

Active Immunization.

In 1888 Richet and Héricourt showed that it was possible to increase the resistance of the rabbit against the staphylococcus by immunization with pure cultures.*

One may immunize either with living or killed cultures or with culture filtrates. Immunization

* Their experiments in protecting and curing other animals with antistaphylococcus serum represent the first attempt made in the direction of passive immunization.

with the bacterial cells must proceed slowly in order to avoid killing the animals. When filtrates containing leucocidin or staphylolysin (hemolysin) are used, antitoxins for these substances are formed. The antistaphylolysin obtained for one strain neutralizes the hemolysin of all strains. The most prolonged immunization with bacterial cells causes no appreciable increase in bacteriolysins.

Protection by Immune Serums. The serum of one who has recovered from a staphylococcus infection, or that of immunized animals, is protective for other animals; 0.1 to 0.2 c.c. of an immune serum given subcutaneously protected mice from a fatal dose of cocci given two hours later, whereas other mice were killed in from 8 to 12 hours. When the serum was given 24 hours in advance of the culture, 0.02 to 0.03 c.c. saved them (v. Lingelsheim, cited by Neisser). The results of Petersen and of Pröscher were similar. In spite of this rather strong protective action, immune serums have little or no curative power.

Properties of Serums. No clearer explanation of the action of the immune serum is given than that afforded by the experiments of Pröscher, who injected guinea-pigs, rabbits and mice with normal and immune serums and followed this 24 hours later with inoculation of the cocci into the peritoneal cavity. Thirty minutes after injection of the cocci the exudate in all animals showed an enormous leucocytosis. At first they were chiefly mononuclears, but later gave place to polynuclears. In the animals which had received the immune serum, massive phagocytosis had occurred, and in the course of an hour very few cocci were extracellular. On

the other hand, practically no phagocytosis had taken place in the animals which had received the normal serum (cited by Neisser). Virulent staphylococci were taken up less readily than avirulent. Such results suggest that the protective power of the serum is due to its ability to stimulate phagocytosis, and this in turn depends on the increased quantity of bacteriotropic substances formed in the serum as the result of immunization (Wright and others).

Vaccination.

In the hands of Wright, vaccination with killed cultures of the staphylococcus has been very successful in the cure of obstinate cases of acne, furunculosis and sycosis barbæ. Bouillon cultures are grown for three weeks and then killed by exposure to a temperature of 60° C. for an hour. In order to control dosage, the vaccine is standardized by estimating the number of bacilli in each cubic centimeter. This is done by mixing equal quantities of the vaccine with normal blood, and, after staining a preparation on a slide, determining the ratio of cocci to erythrocytes. There being about 5,000,000 erythrocytes to the cubic millimeter in normal blood, the number of cocci is readily reckoned from the ratio which was found. From 2,500 millions to 7,500 millions of cocci may be given in an injection. The quantity to be used is determined by the effect which an injection has on the opsonic content of the patient's serum. If a suitable dose has been given, there occurs a short negative phase in which the opsonins are decreased in quantity, and this is followed by a rather prolonged positive phase when they undergo an increase. If too large a dose is given, the negative phase is exaggerated and prolonged. In many in-

"Opsonic Index."

stances it has been noted that improvement and recovery go hand in hand with an increase in the opsonins. The quantity of opsonins present in a serum is expressed by an "opsonic index."*

Agglutination.

The normal serums of man and many animals may agglutinate the staphylococcus, but with no constancy. In one instance human serum agglutinated in a dilution of 1-100 (Kraus and Löw), and normal goat serum in a dilution of 1-50 to 1-400 (Amberger, cited by Neisser). The serums from cases of staphylococcus infection (e. g., osteomyelitis) and of highly immunized animals undergo an increase in the quantity of agglutinins. The agglutination usually is strongest for the homologous strain, and if other strains are agglutinated equally it signifies a close relationship to the homologous strain.

From the fact that only pathogenic strains produce hemolysin and leucocidin, Neisser and

* To obtain the opsonic index human leucocytes, obtained from defibrinated blood, are washed free of serum, and equal parts are added to two equal portions of an emulsion of the staphylococcus. One portion of the emulsion has previously been treated with the serum of the patient for about twenty minutes, and the other portion with normal human serum. The opsonins of the two serums combine with the cocci, rendering them susceptible to phagocytosis (sensitization). After the leucocytes have been in contact with the sensitized cocci for fifteen to thirty minutes, films of the two mixtures are stained with the Romanowsky or a similar stain, which colors both the cells and the cocci. The average number of cocci in say fifty leucocytes on each slide is determined. The average phagocytosis in the patient's serum divided by that in the normal serum gives the opsonic index. For example, if the former showed an average of 10 cocci to each leucocyte and the latter an average of 5, the index is 2. The degree to which the opsonic index can be raised by the immunization varies. In one instance it was increased from .8 to 2.6; in another case which reacted less vigorously it was raised from .87 to .95.

Wechsberg considered them specifically different from non-pathogenic strains. This view is borne out by the results obtained with the agglutination test. Serums obtained by immunization with pathogenic strains have a much higher agglutinating power for these strains than for non-pathogenic varieties, and the converse is also true. There are, however, many variations in the agglutinability of the members in each group, a fact which indicates variations in the receptor complex of the different strains. It has been suggested that a polyvalent serum obtained by immunization with a sufficient variety of pathogenic strains will be efficient in differentiating the latter from non-pathogenic varieties by means of the agglutination test.

Wright, noting an increase in the agglutinating power when patients are treated by his method, considers that this increase is an index of the immunity which is established.

IV. MICROCOCCUS CATARRHALIS.

For some years diplococci resembling the gonococcus and the meningococcus morphologically and in staining reactions have been found in the sputum by a number of observers, and to this coccus Pfeiffer gave the name of *Micrococcus catarrhalis.* It is frequently found in the respiratory passages in influenza-like infections and other inflammatory conditions, and occasionally in lobular pneumonia. It may be associated with the influenza bacillus or the pneumococcus. Among 140 cases of diseases of the respiratory passages Gohn and H. Pfeiffer found it eighty-one times, and M. Neisser demonstrated it in sixteen cases of whooping-

cough, in one of measles and scarlet fever, and in two of diphtheria. It loses significance in relation to these diseases, however, since Jündell found it frequently in the mucus of the normal trachea, and Weichselbaum cultivated it frequently from the healthy nasal fossæ. According to Gohn, Pfeiffer and Sederl, "The *Micrococcus catarrhalis,* without the association of other microbes, is able to cause bronchitis and pneumonia with the clinical type of pneumonia due to the pneumococcus. The symptoms caused by the *Micrococcus catarrhalis* do not form a clinical type. They resemble infections by the pneumococcus or the bacillus of Pfeiffer (Influenza)" (cited by Bezancon and de Jong). Others are not so positive concerning the pathogenic properties of the organism. Its etiologic rôle is not yet well established. It has little pathogenicity for animals, although peritoneal and pleural infection is possible in guinea-pigs.

It differs from the gonococcus and meningococcus in certain cultural characters.

V. GONORRHEA AND OTHER INFECTIONS WITH THE GONOCOCCUS.

The Gonococcus.

A. Neisser discovered the gonococcus in 1879, cultivated it in 1884, and demonstrated its specific relation to gonorrhea by the inoculation of pure cultures into the human urethra. It is a diplococcus, young pairs having a figure-of-eight contour, whereas older pairs show a typical biscuit or coffee-bean shape. The organism is non-motile, has no flagella and forms no spores. It can be cultivated only on media which contain serum, ascitic or a similar fluid. Its failure to stain by Gram's method is of great diagnostic importance

in the examination of urethral discharges; other organisms resembling the gonococcus are found in the urethra and vagina with great rarity. The reaction loses its differential value in the examination of secretions of the nose, mouth, and, to some extent, of the conjunctiva, where the meningococcus and the *Micrococcus catarrhalis* may be encountered.

Phagocytosis.

In the purulent stage of a gonorrheal infection the cocci are found almost entirely within the leucocytes, whereas in earlier stages, when the discharge is slight and of a mucous character, and also during convalescence, when the secretion again becomes mucous, they are largely extracellular. They are never within the nuclei. The process is one of active phagocytosis in which the cocci play a passive rôle. They occur not only on the surface of the epithelium, but penetrate between and beneath the epithelial cells, and even into the adjacent connective tissue.

Cultivation and Resistance.

In culture media growth is slow and scant, and cultures rarely live longer than one or two weeks, unless they are transplanted to suitable fresh media. On the latter they may be carried through many generations without losing their virulence. When dried they die very quickly, but may live for some hours on linen (towels) or the skin, and for twenty-four hours in warm water. They are very susceptible to temperatures above 42° to 43° C. and show very little resistance to antiseptics, particularly the silver salts.

Toxicity and Virulence.

The gonococcus secretes no soluble toxin, but contains an endotoxin or toxic "protein" which causes local and general symptoms in both man and animals. Dead cultures produce an inflamma-

tory exudate in the peritoneal cavity of guinea-pigs and mice, resulting in death if the dose is sufficiently large, and when injected into the urethra of man a temporary inflammation results. An actual infection of any sort can not be produced in animals; the cocci are killed without being permitted to proliferate. The endotoxin (gonotoxin) is fairly resistant to heat, being destroyed only after prolonged exposure to a temperature of 100° C.

Susceptible Tissues.

In man the mucous membranes and endothelial surfaces are more susceptible to infection than other tissues. The urethra of male and female at all ages, the conjunctiva in the new-born, the vagina, uterus and tubes are probably the most susceptible. Less susceptible are the vagina in older women, especially those who have borne children; the bladder, and in adults the conjunctiva. It is remarkable that there are so few cases of gonorrheal ophthalmia in adults, considering the opportunities for infection. Infection of the mouth, nose and tear sacs is extremely rare. Extension from the urethra to adjacent structures takes place either by way of the surfaces, as in involvement of the prostate, epididymis, glands of Bartholin, uterus, tubes, ovaries, peritoneum, bladder and kidneys, or by way of the lymphatics as in infections of the periurethral tissue or cellular tissue of the pelvis. Usually infections of the bladder and kidney, and not infrequently of the prostate, Fallopian tubes and pelvic tissue are of a mixed character (staphylococcus, streptococcus), but not necessarily so. Arthritis, tendovaginitis, endocarditis, which usually is vegetative but may be ulcerative, are the more common metastatic complica-

tions. Less frequent are pericarditis, pleuritis, subcutaneous abscesses and iritis. As to whether the cutaneous phenomena sometimes seen are due to metastases or are of purely toxic origin seems to be undetermined. The blood stream may be infected by way of the lymphatics or local blood vessels (gonorrheal thrombosis).

The influence of the enormous phagocytosis of the cocci on the course of gonorrhea is unknown. Since the ingested cocci usually have a typical form and stain well, it would seem that they resist the action of the leucocytic ferments. Likewise the nuclei of the leucocytes usually stain well, hence there is no evidence of a marked toxicity of the cocci for these cells. The mechanical imprisonment of the organisms by the leucocytes may be of influence in localizing the infection.

Urethral Changes.

During the course of gonorrhea "there takes place a pronounced metaplasia of the epithelium in which the cylindrical cells are changed into a more cuboidal and even pavement form." Following this change the gonococci are limited to the surface of the altered epithelium and penetrate more deeply only in the vicinity of the glands and crypts. "Eventually the gonorrheal process is limited to such isolated points and the gonorrhea thereby enters into a chronic stage" (observations of Finger, cited by Neisser and Scholtz).

Chronic Gonorrhea.

The conditions which cause the subsidence of acute gonorrhea and allow it to persist as a chronic infection have been the subject of much speculation, unproductive for the most part. It is not due to a decrease in the virulence of the cocci since their original infectiousness is retained for others; nor does the local resistance of the mucous

membrane reach a high point, since reinfection, or better "superinfection" is possible at any time. A man suffering from chronic gonorrhea and having infected his wife, may again be infected by his wife when the gonorrhea of the latter has become subacute or chronic. It has been suggested that the condition in chronic gonorrhea may be one of "mutual habituation between the mucous membrane and the gonococcus," i. e., a habituation between this particular mucous membrane and this particular gonococcus. Because of prolonged existence under unvarying conditions, the growth energy of the organism may have become less, whereas, if it is placed in a slightly different medium (transference to another individual), its growth energy (ability to proliferate), becomes augmented, and reinfection of the original host with the same strain becomes possible.

It has often been noted that subsequent attacks run a milder course than the primary infection, but susceptibility is always present.

Immunity. Mendez, Calvino, and also de Christmas have immunized with the coccus or toxic substances prepared from it. By growing the organism in serum bouillon de Christmas prepared a toxin, the toxicity of which was tested by intracerebral injections in the guinea-pig. Immunization of the guinea-pig resulted in a serum with antitoxic properties. Corroborative work has not been published.

VI. EPIDEMIC CEREBROSPINAL MENINGITIS.

Microbes Causing Meningitis. Acute inflammation of the meninges may be caused by a number of micro-organisms: *Micrococcus meningitidis*, also called the *Diplococcus*

intracellularis meningitidis, or briefly the meningococcus; *Diplococcus pneumoniæ; Streptococcus pyogenes; Staphylococcus pyogenes; Bacillus influenzæ; Bacillus pneumoniæ; Bacillus typhosus; Bacillus coli communis; Bacillus mallei; Bacillus pestis.* The first two of this number, the meningococcus and the pneumococcus, in addition to causing sporadic cases, also produce more or less extensive epidemics of so-called primary meningitis. That the pneumococcus may also cause meningitis secondary to pneumococcus infections in other parts of the body has been mentioned. Also the meningitis caused by the other pyogenic cocci usually is secondary to some other suppurative focus, often the middle ear; that caused by the organisms of typhoid, glanders, plague and influenza occurs during the course of the diseases caused by the corresponding micro-organisms.

Micrococcus Meningitidis.

Previous to 1887 diplococci resembling the pneumococcus had been found in the exudate in cases of cerebrospinal meningitis by Foà and Bordoni-Uffreduzzi, by Fraenkel and others. Weichselbaum made similar observations during the same year, and in addition described six cases in which a diplococcus of another nature was present in pure cultures. To the latter he gave the name of *Diplococcus intracellularis meningitidis.* Extensive observations by others, both in Europe and America (Councilman, Mallory and Wright, and others), revealed the presence of the last-named organism in many instances, and showed that it is the most common cause of epidemic cerebrospinal meningitis.

The meningococcus resembles the gonococcus closely in that it is usually found in biscuit-shaped

pairs, nearly always within pus cells, and does not stain by Gram's method (Weichselbaum). It is properly to be called a micrococcus since it divides in two transverse directions (Albrecht and Gohn); tetrads, small groups and short chains are sometimes seen. However, it forms no striking chains, is non-motile and produces no spores. Growth may be obtained on some of the ordinary media (glycerin agar), in which the organism differs from the gonococcus, but a medium which contains blood or serum is much more favorable. It is an obligate aërobe, grows best at the body temperature and virulence is soon lost under artificial conditions.

Resistance. It produces a membrane on meat broth with clouding of the medium. Viability is retained only for a few days at room temperature. When dried on paper and exposed to the sunlight it lives no longer than twenty-four hours, in a dark room seventy-two hours (Councilman, Mallory and Wright). It is killed by a temperature of 65° C. for thirty minutes (Albrecht and Gohn).

Virulence; Endotoxin. The meningococcus has little virulence for animals. When injected in sufficient quantity into the peritoneal or pleural cavity of white mice death results in from twenty-four to forty-eight hours, but not when given subcutaneously. Meningitis may be produced by subdural injections, but the disease does not resemble the epidemic meningitis of man. So far as is known at the present time the organism does not produce a soluble toxin, but possesses rather an endotoxin.

Infection Atria. Although the disease is usually spoken of as a primary meningitis, there is reason to believe that it is secondary to an acute rhinitis or acute in-

flammation of the accessory sinuses or middle ear, in many instances. From these places the coccus may readily reach the meninges by way of the lymphatic channels. It has been found repeatedly in the noses of those who were associated with cases of the disease; in such cases an acute rhinitis may be present without the subsequent development of meningitis. Clinical histories show that the infection commonly is preceded by acute rhinitis. The inflammation in the meninges is always cerebrospinal in its distribution and is characterized by a purulent or fibrino-purulent exudate in which the diplococci are present in varying quantities. Diagnosis may often be established clinically by the microscopic or cultural examination of the cerebrospinal fluid which is removed by lumbar puncture.

Complications and Other Infections.

Acute encephalitis, acute bronchitis, lobar pneumonia and acute arthritis have been observed as complications, in which organisms resembling the meningococcus have been found in a number of instances. An accompanying bronchitis, lobar or lobular pneumonia may be caused by mixed infection with other organisms (pneumococcus, streptococcus, staphylococcus). Since it would be difficult to explain some of these complications except on the basis of metastasis, it seems very probable that the organism reaches the blood stream. Micrococci resembling the meningococcus have been found in acute bronchitis, rhinitis, lobular pneumonia and conjunctivitis, in the absence of cerebral involvement, and it is possible that it may be the cause of independent inflammations in these tissues. Weichselbaum, however, is inclined to doubt the identity of such organisms with the

meningococcus. Particularly in cases of bronchitis and lobular pneumonia the coccus may be confused with the *Micrococcus catarrhalis* of Pfeiffer, with which it is identical morphologically.

The extent to which the meningococcus is a normal inhabitant of the nasal mucous membrane is unknown.

Transmission and Contagiousness.

Since the organism seems to be excreted chiefly or only with the nasal discharges, the latter probably are important for transmission of the infection. Because of the low resistance of the organism to desiccation and light, transmission probably is a fairly direct one. This is suggested also by the occasional occurrence of epidemics in institutions. Contagiousness is of a rather low order; this is indicated by the distribution of the 111 cases observed by Councilman, Mallory and Wright in Boston, the city being somewhat diffusely infected with very little tendency of the disease to occur in groups of individuals or in several members of a family.

The desirability of avoiding contact with the infected is evident; special prophylactic measures are not known. In the presence of an epidemic the treatment of rhinitis with local antiseptics would suggest itself.

Susceptibility and Immunity.

Children and young people are particularly susceptible to both epidemic and sporadic infections with the meningococcus. Exposure incident to the cold and variable weather of the winter and spring, in which seasons the disease prevails, may be influential in lowering resistance. Second attacks are rare, Councilman, Mallory and Wright collecting only five such examples from the literature. Lipierre immunized animals with cultures and

with a toxin, the latter being a glycerin extract of old cultures. Their resistance to infection was said to be increased, and the serum of highly immunized animals was antitoxic, preventive and curative for other animals. Corroborative work is lacking. According to Davis, the serum in cases of epidemic meningitis shows an increased bactericidal power for the coccus on the thirteenth day of the disease; the agglutinins which develop probably persist for some time, but are little above the normal after two and one-half years.[1] Fairly strong agglutinins may be obtained by the immunization of rabbits (Jäger and Albrecht and Gohn).

VII. INFLUENZA.

Influenza occurs sporadically and in epidemics of greater or less proportions. Its extreme contagiousness is shown by the striking rapidity with which it spread over the whole civilized world in the epidemic of 1889 and 1890, leaving behind it

1. The conclusions of Dr. Davis are as follows: In five cases of epidemic cerebrospinal meningitis, the meningococcus (Weichselbaum type), was obtained in every case from the cerebrospinal fluid, and in one case from the nose and sputum by cultures. In the other four cases Gram-negative diplococci suggestive of either meningococcus or *Micrococcus catarrhalis* were seen in smears, but were not recovered in cultures. Agglutination of meningococcus by the serum of patients with meningitis occurs in a dilution of 1-5 or higher. The meningococcus grows in some defibrinated normal bloods, but not in others, there being thus an interesting individual variation. In the blood of three meningitis cases it did not grow. Normal human serum is distinctly bactericidal toward the meningococcus. This property is increased in sera of meningitis cases, and is diminished, but not entirely destroyed by heating to 60 C. for thirty minutes. Cerebrospinal fluid acts in much the same way as heated serum. The opsonin content of the blood does not appear to be altered during the course of epidemic meningitis. Normal cerebrospinal fluid does not contain opsonin for meningococci. —*Jour. of Infectious Diseases*, 1905, vol. ii.

a trail of lesser epidemics which have prevailed up to the present time.

Bacillus Influenzæ. During the epidemic just cited a number of organisms were erroneously described as the cause of the disease. In 1892, however, Pfeiffer discovered a minute bacillus which he found constantly and in large numbers in the sputum of influenza patients only. The observations of Pfeiffer have been confirmed by a large number of investigators, and the organism, *Bacillus influenzæ*, is now accepted as the cause of the disease. It is one of the smallest of bacteria (0.2 or 0.3 by 0.5 microns), is non-motile and forms no spores. A medium containing blood or hemoglobin is essential for its artificial cultivation, and even under the best conditions it grows meagerly and slowly. A number of bloods, but particularly those of man and the dove, favor its growth. It is a strong aërobe. The organism is best stained by a dilute solution of carbol fuchsin (1 to 10), and, like the plague bacillus, exhibits polar staining, i. e., the ends stain more deeply than the central portion.

Symbiosis. When the staphylococcus and some other organisms are grown in mixed culture with the influenza bacillus, the latter is stimulated to a more vigorous growth. According to Jacobsohn, killed cultures of the streptococcus greatly increase the virulence of the influenza bacillus when the mixture is injected into animals.

Pseudo-Influenza Bacilli. Pfeiffer designates as pseudoinfluenza bacilli a number of influenza-like organisms which have been found in man and animals. They have the morphology of the influenza bacillus, are a little larger, and also prefer a medium which contains hemoglobin, but since some of them occur in ani-

mals which are known not to be susceptible to influenza, it is concluded that they can not be identical with the influenza bacillus. The influenza-like bacillus which Jochmann and Krause consider as the cause of whooping-cough, may be mentioned in this connection.

Resistance and Virulence.

The resistance of the bacillus to desiccation, sunlight and unfavorable temperatures is very low. It dies in from twenty-four to thirty-six hours at room temperature, when contained in sputum, and lives for about thirty-two hours in hydrant water (Pfeiffer). It is not highly virulent for experiment animals, although a condition said to resemble influenza has been produced in monkeys by placing pure cultures on the nasal mucous membrane. Fatal infections may be produced by intravenous inoculation of the bacillus into monkeys and rabbits, and killed cultures produce a fatal intoxication in rabbits. Virulent cultures in sufficient quantity produce fatal peritonitis in guinea-pigs. Since the bacilli seem not to proliferate when fatal quantities are injected intravenously into rabbits, and since fatal intoxication, without the occurrence of bacteriemia, may take place when a tracheal infection is induced in the ape (Pfeiffer), it is concluded that the toxic phenomena of influenza are due to the absorption of bacterial toxins from the mucous surfaces. A soluble toxin has not been obtained in culture media. The organism is a facultative pus producer.

Distribution in the Body.

So far as is known the influenza bacillus is excreted only with the secretions of infected surfaces, i. e., from the upper respiratory passages, conjunctiva, ear, etc. The belief, commonly held, that the influenza bacillus does not enter the cir-

culation probably is erroneous. That metastatic infection is possible, by way of the lymph or blood channels, is shown by the occurrence of infiuenza meningitis, and, rarely, of influenza peritonitis (Hill and Fisch). According to Jehle, the influenza bacillus invades the blood very frequently in some of the acute exanthemata. It was found in the blood in 22 out of 48 cases of scarlet fever, in 15 of 23 cases of measles, and in 5 of 9 cases of varicella (cited by Hektoen). Hence, these diseases would seem to create conditions favorable for invasion by this bacillus. When the bacilli reach the blood they probably are killed quickly. It is probable that the ordinary nervous phenomena of the disease are due to intoxication rather than to actual infection of the nervous structures. As to whether the symptoms of so-called intestinal influenza are due to an invasion of the intestines by the bacilli or to a specialized action of circulating toxin seems not to have been definitely settled. There certainly is abundant opportunity for infection of the intestines in cases of bronchial influenza. In the bronchitis of influenza the organisms are found in large numbers in the smaller bronchial tubes, both free and within leucocytes, hence, in searching for the bacilli clinically it should be certain that the sputum examined represents the bronchial exudate. In influenza pneumonia, which usually is of the lobular type, the bacilli, mixed with pus cells and contained in them, are found in large numbers in the alveoli. Pure cultures of the bacillus have been obtained from cases of conjunctivitis, and they occur not infrequently in middle-ear complications which develop during the course of the disease. Influ-

enza conjunctivitis sometimes occurs in epidemic form, particularly in institutions and schools.

Mixed Infections.

Pneumonic foci which develop during influenza frequently show the pneumococcus, and sometimes the streptococcus or the bacillus of Friedlander in addition to the influenza bacillus, and similar mixed infections occur in pleurisy and in middle-ear disease. Influenza may be superimposed on other infections; individuals suffering from pulmonary tuberculosis are particularly susceptible to influenza, and in them the prognosis is unfavorable.

Transmission, Infection Atria and Prophylaxis.

The disease is transmitted directly from man to man and, chiefly, it is supposed, by means of infected droplets of sputum which are expelled in coughing and sneezing. Obviously kissing affords opportunity for infection. Infection by indirect contact is of less importance because of the rapid death of the bacillus after it leaves the body, but living germs may well be disseminated by soiled handkerchiefs or other contaminated linen. Dust infection possibly is of minor consequence. Chronic influenza in which the bacilli may persist in the bronchi for weeks, and cause recurrent acute attacks, is of importance for the maintenance of an epidemic. In tuberculous cavities the bacilli may flourish for long periods.

Primary infection takes place in the upper respiratory passages, and the disease extends readily from one surface to another, as from the nose to the pulmonary tissue. Infection of the ear usually is a complication of pharyngeal or pulmonary infection. Occasionally an influenza conjunctivitis is found without other localization. "Primary" infection of other organs, as the brain and perito-

neum, are metastatic, although the original focus or atrium may not be observed.

Little or nothing can be done in the way of general prophylaxis. Washing of the nose and mouth with antiseptics during an epidemic may reasonably be practiced, but with what success is uncertain. The aged and those of low vitality should avoid exposure to infection, for in them the severer complications, such as pneumonia, are more likely to occur. When influenza conjunctivitis appears epidemically in schools, the latter should be closed or the infected children excluded.

Immunity, Susceptibility and Recurrences.

Although little or nothing is known concerning the possibility of a natural immunity in man, experience teaches that he is, on the whole, very susceptible. The belief expressed by some that nursing children are less susceptible than older people seems to have some foundation, although it is well known that they are not entirely immune. Influenza is sometimes cited as an infection in which one attack creates a predisposition for a second, but the truth of this is doubted by many who have had extensive experience with the disease. Wutzdorff, in a study of the epidemic which prevailed in Germany during 1891-92, finds in the small number of cases, the irregularity of their distribution, and comparative exemption of rather large districts, reasons for believing that one attack confers a degree of acquired immunity; that is to say, the population had been so thoroughly infected (*durchgeseucht*) during the preceding year or two that comparatively few remained who were susceptible, although the disease itself appeared to be more malignant than in the previous year (cited from Beck). However, the occurrence of second

attacks shortly after the first, and of repeated infections in some individuals indicate that acquired immunity is of short duration. The aged, those of low vitality, and those with pulmonary tuberculosis, have low resistance to infection.

Serum Properties.

Although Delius and Kolle were able to produce a slight increase in the resistance of guinea-pigs by the intraperitoneal injection of cultures, nothing like a well-marked immunity was obtained; nor did the serum of immune animals or convalescent man show increased protective power for other animals. Slatinéano, however, obtained serum of some protective value for guinea-pigs, by the immunization of rabbits and guinea-pigs, but it had no curative effect. The results of Cantani were similar, and both observers noted the development of bactericidal power, as determined by the Pfeiffer reaction, and of agglutinins. At the present time there seems little to hope from vaccination.

There is said to be some increase in agglutinins in man as a consequence of infection. The agglutinating power of the serum of an immunized animal may be as high as 1 to 500 (Cantani).

VIII. SOFT CHANCRE.

The independence of soft chancre and syphilis, and the infectiousness of the former by inoculation with the purulent secretions of the ulcers, were established long ago. Rollet found that filtered pus lost its infectiousness.

Bacillus of Ducrey.

A large number of observers had found bacteria of one kind or another in the pus and in stained sections of the walls of the ulcers, and probably some of them (e. g., Unna), had seen the bacillus

which Ducrey described (1889) and later cultivated, and which is now proved to be the cause of the disease. The bacillus is very small (0.4x1.5 microns), is non-motile and shows polar staining. It resembles the plague bacillus in form, but is somewhat smaller, and does not show the extensive involution forms of the latter. In the ulcer it lies singly, in small groups, or more characteristically in the form of bands, made up of two or more parallel chains, which infiltrate the wall of the ulcer. Large numbers are often found in the polymorphonuclear leucocytes of the pus, particularly at an early stage of the lesion (Kroefting). Great difficulty was encountered in cultivating the bacillus, and Ducrey's first success was obtained with a medium which contained human skin. It has since been cultivated on agar which contains the blood or serum of man, rabbit or dog. Himmel attempted to cultivate it in the fresh defibrinated blood of the guinea-pig, but was unsuccessful because the bacilli were phagocytized by the leucocytes (Babes).

An ulcer resembling that of soft chancre may be produced in the ape, and also in the cat, by the inoculation of pure cultures. Didey reinoculated man, successfully, from the ulcers of the cat. When living cultures are injected into the guinea-pig (peritoneal cavity, subcutaneous tissue, dura mater), the bacilli are quickly taken up by leucocytes and digested (Himmel). Himmel reports having so decreased the resistance of guinea-pigs by peritoneal injections of lactic acid that they became susceptible to infection. After two or three passages the culture became so virulent that

fatal bacteriemia was caused without previously lowering the resistance of the animals.

In man the infection is transmitted to the inguinal lymph glands, but never becomes general.

One attack in man does not confer lasting immunity. Spontaneous recovery occurs, but its cause is not known. Inasmuch as the bacilli are found within leucocytes, phagocytosis may be a factor in recovery. The readiness with which the autoinoculation of adjacent skin takes place, even after the disease has existed for some time, suggests that general immunity is not established.

IX. BACILLUS OF FRIEDLANDER AND OTHER MEMBERS OF THE CAPSULE-FORMING GROUP.

Capsulated Bacilli.

The bacillus of Friedlander, or *Bacillus pneumoniæ,* is the type of a rather large group of bacteria, called the Friedlander group, or the group of *Bacillus mucosus capsulatus.* In addition to the ability to produce a mucus-like capsule or envelop, they have in general the following characteristics (Abel): short, plump rods, varying in their proportions, having no motion, no flagella, no spore formation, and not staining by Gram's method. They form mucus-like masses in cultures, do not liquefy gelatin and are facultative anaërobes. They are widely distributed in nature, vary from innocuousness to extreme pathogenicity for animals, are rarely found in the mouth, nose and bronchi normally (bacillus of Friedlander), one type being a normal inhabitant of the intestines, especially in children (*B. lactis aërogenes*). Perkins has been able to classify the members of this group on the basis of their fermenting powers for lactose and saccharose. He found their viru-

lence for animals, immunization and agglutination tests, too variable to serve as bases for classification. In man three members of the group—they may be the same organism or variations of a type—are of interest from the standpoint of infection: Bacillus of Friedlander, the bacillus of rhinoscleroma and the ozena bacillus.

Pneumonia Caused by Friedlander's Bacillus.

In 129 cases of acute inflammation of the lungs, Weichselbaum found the bacillus of pneumonia nine times, twice with streptococci and once with the diplococcus of pneumonia. The organism causes lobular pneumonia more frequently than lobar. The homogeneous non-granular surface, and the large amount of fluid of a viscid or mucous consistence, are characteristic anatomic features. The alveoli contain massive numbers of the bacilli. The bacillus of Friedlander is found also as the cause of pyelitis, cystitis, pyelonephritis, serous or purulent pericarditis, pleuritis and meningitis, which may be accompanied by brain abscesses. Meningitis when produced by this organism usually or always is secondary to infection in other parts of the body by the same organism (middle ear and accessory sinuses of the nose).

Rhinoscleroma and Ozena.

An organism of the Friedlander type is found with few exceptions in the tissues in rhinoscleroma, and by many is considered as the cause of the condition. A similar organism is found constantly in the secretions and crusts in ozena.

Antiserums of distinct power have not been obtained for members of the group. Prolonged immunization with some strains yields an agglutinating serum of low value. The agglutination reaction is of no value for identification of the different members of the group, nor for clinical diagnosis.

X. RELAPSING FEVER.

The Parasite.

In 1868 Obermeier discovered in the blood of patients suffering from relapsing fever, "very fine threads exhibiting motility"; these "threads" have since been known as the *Spirocheta obermeieri.** and are recognized as the cause of the disease. They are very thin (about 1 micron), from 10 to 40 microns in length, and of spiral form. Three types of motion are described: a screw-like, a forward and backward movement and a lateral bending. They are found only in the blood and blood-forming organs. They disappear from the blood with remarkable rapidity at the time of crisis, although they may be found in the spleen one or two days later.

The organism has not been grown artificially, but it may be kept alive for a number of days in the blood or serum of patients. As the micro-organisms die agglomerations are formed and they undergo granular changes.

Transmission

The organism is not found in Nature, and, since it occurs only in the blood of the sick, it has long been assumed that infection can be accomplished only by the inoculation of infected blood.

---

* This organism is sometimes called a spirillum, incorrectly. The spirillaceæ, Migula's third family under the Order of Eubacteria, comprises organisms with these characteristics: "Cells which are twisted screw-fashion or represent a segment of a spiral. Division takes place only in one direction of space after the cell has elongated." The difference between spirillum and spirochæta is shown by the following: "3. Genus: Spirillum. Cells rigid, with polar tufts, for the most part bent in the form of a half-circle, as organs of locomotion. 4. Genus: Spirochæta. Cells with snake-like bending, organs of locomotion unknown." Although Migula classes this organism with the bacteria, there is some ground for considering it protozoon in nature.

The parasites have been demonstrated repeatedly in bedbugs which are found on the mattresses of the sickbed, and monkeys have been infected by inoculating them with the blood found in the bodies of these insects, and by the bites of the latter (Tictin). It is said that they may remain alive in bedbugs for as long as thirty days. It is not altogether excluded that other vermin also transmit the disease.

The spirocheta does not appear in any of the excretions, unless they happen to be of a bloody character.

Certain monkeys, those belonging to the slender-nosed family (*Catarrhinæ*), may be infected by injecting the blood of patients, provided the blood used is taken during the paroxysm, i. e., at a time when the microbes are known to be in the blood. Monkeys do not contract the disease under natural conditions. Other animals are not susceptible. The incubation period in man usually is from five to seven days, and in monkeys from one and one-half to four days. Cloudy swelling of the parenchymatous organs, ecchymoses and infarcts of the spleen and kidneys are found in fatal cases.

Prophylaxis consists in isolation of the patient, cleanliness, and the destruction of vermin, especially bedbugs.

Relapsing fever occurs in various races of man, and so far as known none are immune. Osler states that in the United States the disease has not been seen since 1869, when it was epidemic in New York and Philadelphia. The natural immunity of other animals is referred either to phagocytosis or to normal bacteriolysins, but the conditions probably are not thoroughly understood.

Phagocytosis and Bacteriolysins.

As stated above, a remarkable feature in the course of the disease is the rapidity with which the micro-organisms disappear from the blood at the time of the crisis. Metchnikoff refers this to phagocytosis by the microphages, which undergo a progressive increase during the paroxysm and decrease after the crisis. Very little phagocytosis appears to take place in the circulating blood, but in the spleen many spirochetæ are found within polymorphoneuclear leucocytes. Tictin also found them in the parenchymatous cells of the kidney, liver and lungs. Phagocytosis is most marked at or near the time of the crisis. According to Metchnikoff, relapse or reinfection is accomplished by spirochetæ which again invade the body from the spleen.

Russian observers have studied the development of a specific bactericidal power in the serum of the sick and in animals which were immunized by the injection of infected blood from man. Inasmuch as the organism can not be cultivated, bactericidal tests must be performed with the organisms as they occur in the blood or serum of the patients, and Gabritschewsky has devised a technic for this procedure.

Technic.

A drop of serum from an immune animal or a convalescent patient is mixed on a slide with a drop of serum which contains the spirocheta, the latter serum being taken from a patient during an attack. The preparation is sealed under a coverglass and examined at intervals, and the death of the organisms is determined by their loss of motility. It is said that the bactericidal power of human blood following infection, and that of immunized animals, is increased.

Active and Passive Immunity.

In view of the facts that three or more relapses may occur and that reinfection is possible at a later period, it seems probable that man does not readily acquire immunity to the infection, although second and third relapses are said to be lighter than the first. Monkeys which have been artificially infected several times acquire some resistance to the disease. The view of Metchnikoff that the spleen is essentially involved in recovery and immunity seems to have been disproved by the experiments of Tictin, who found that splenectomy had no influence on recovery or the development of immunity.

The serum of convalescents affords a certain degree of protection to the monkey (Gabritschewsky). Löventhal utilized the serum of immunized horses in the treatment of the disease in man, and reported a decrease in the number and severity of relapses. The action of the serum has been referred both to its content in bactericidal antibodies, and to its ability to stimulate phagocytosis.

Melkich states that agglutinins are formed and that they appear on from the third to the fifth day of the disease.

A rapidly fatal disease of geese, spirocheta septicemia, or spirillosis of geese, is caused by an organism which resembles the spirocheta of Obermeier, and a similar infection has been noted in chickens in Brazil and in cattle in the Transvaal.

GROUP IV.

Infectious diseases which usually are chronic, but may run acute courses. They are characterized by marked local tissue changes, which exert a limiting influence on the processes, and include the infectious granulomata, excepting syphilis. Infection produces little or no immunity. In some instances the prolonged immunization of animals induces increased resistance to infection (tuberculosis); in other instances this has not been determined, or is difficult of determination because of the non-susceptibility of experiment animals to the corresponding infections. The serums of immunized animals, in so far as this subject has been investigated, show little or no protective or curative power.

I. TUBERCULOSIS.

Klemke, in 1843, but more particularly Villemin, in 1865, demonstrated the infectiousness of tuberculosis by animal experiments, and these results were substantiated later by such investigators as Klebs, Chauveau, Baumgarten and Conheim. Baumgarten first saw the tubercle bacillus in sections of tuberculous material from which the tissue cells had been dissolved by potassium hydroxid, and at almost the same time Koch succeeded in demonstrating its presence in all tuberculous lesions by a special staining method. He eventually obtained the organism in pure cultures with which he again produced tuberculosis in experiment animals.

The tubercle bacillus is an obligate aërobic parasite, has the form of a slender, non-flagellated rod,

Characteristics of the Bacillus.

often slightly curved, from 2 to 4 microns long and from 0.3 to 0.5 microns broad. In stained and even in unstained specimens, when properly treated, a number of spherical, oval or elongated clear spaces can be seen which Koch at one time thought to be spores. They are now considered either as vacuoles, or as representing some form of degeneration or reserve nutritious material. Spore formation is uncertain. The organism is supposed to possess a membrane which may be responsible for its strong resistance against heat and desiccation. Feinberg speaks of a nucleus (?) which may be demonstrated by a modified Romanowsky stain. The organism shows many variations in its morphology under different conditions. It often exists in isolated clumps, either in cultures or in tissues, and may be excreted as such in the urine. In certain cultures and sometimes in animal tissues it grows in the form of longer or shorter branching threads, in this respect resembling actinomyces. This last occurrence has led a number of authorities to class the tubercle bacillus as a streptothrix, while others would give it an intermediate position between true bacteria (schizomycetes) and the streptothrix (a hyphomyces). Oval or spherical degeneration forms, the capsules or corpuscles of Schrön, are found in advanced tuberculosis of the lymph glands and other organs in which there is a great deal of necrosis.

Staining Properties.

The tubercle bacillus is one of a group of organisms which are said to be "acid fast" in their staining properties. When stained with the carbol fuchsin of Ziehl and subjected to the action of mineral acids in dilute solutions the fuchsin is not removed. After counterstaining with methylene

blue, the tubercle bacilli appear red, whereas other organisms, not "acid fast," are stained with the methylene blue. It is not difficult to recognize the bacilli in sections of tissue when the proper technic is used, although the search is at times a laborious one. In old processes the organism often can not be recognized, and recourse to animal inoculation may be necessary in order to demonstrate the existence of tuberculosis.

Cultivation.

It is ordinarily a difficult task to obtain the tubercle bacillus in pure culture, the technic of which we need not consider. Even under the best conditions growth is very slow, and may not be recognizable to the naked eye for from six to ten days. Coagulated serum of the cow to which has been added from 2 to 4 per cent. glycerin is the most favorable culture medium. Good growth occurs also in glycerin agar, in glycerin bouillon and on potatoes. The optimum temperature is 37° C.; growth does not occur above 42° C. nor below 30° C. When a small amount of culture is planted on the surface of glycerin bouillon it proliferates slowly to form a heavy membrane. In time this growth sinks from its own weight and a new membrane forms. This process continues until large masses have accumulated at the bottom of the flask.

Resistance.

In its resistance to desiccation the tubercle bacillus is exceeded only by spore-forming organisms; it lives approximately for three months in dried sputum which appears to form a protective coating about it. Direct sunlight destroys it in a few hours at the most, whereas diffuse light kills it only after from five to seven days (Koch). It is said that the guinea-pig when exposed to sunlight

withstands tuberculosis for a longer time than one which is kept in the dark. Roentgen rays are bactericidal for the organism, killing it in about one hour (Rieder). Under moist heat a temperature of 55° C. kills it in from four to six hours, 60° C. in one hour, 70° C. in from ten to twenty minutes, 80° C. in five minutes, from 90° to 95° C. in from one to two minutes. When embedded in sputum it is more resistant, five minutes being required to kill it at the boiling temperature. Corrosive sublimate is not a good disinfectant in this case, inasmuch as it produces an albuminous precipitate around the organism which prevents penetration of the sublimate. Five per cent. carbolic acid added to equal parts of sputum kills the bacillus in twenty-four hours. Formalin vapor is a good disinfectant for dry, but not for moist sputum. Iodoform is not a good disinfectant, in spite of its beneficial influence on the infectious process. The resistance of the bacillus to gastric digestion has an important bearing on the occurrence of infection in the intestinal tract. The gastric juice of the dog, in one instance, failed to kill the bacillus after six hours' exposure, although it had the power of prohibiting proliferation.

Virulence. The bacillus of human tuberculosis, although fairly constant in its virulence, may be attenuated by various means. Its prolonged existence in putrid sputum decreases its virulence and a similar decrease occurs on potato, in old cultures or in those which contain iodoform, boracic acid and some other substances. Inoculation with such cultures produces a chronic form of tuberculosis in animals which may heal. In other instances cultures which have grown on artificial media for many years retained their original virulence.

The organism contains about 90 per cent. of water. One-fourth of a dried bacterial mass may be extracted as a wax-like or fat-like substance by a mixture of alcohol and ether. The acid-fast staining property of the bacillus depends on this substance. The remaining portion of the mass, consisting largely of proteins, which may be extrated by dilute alkalies, contains a toxic nucleo-albumin. Cellulose, representing a portion of the capsular substance, is also found in the residue.

Toxic Products.

Killed cultures when given subcutaneously produce necrosis, abscesses, caseation, marasmus, and a subnormal temperature. When given to rabbits and guinea-pigs intravenously they cause rapid emaciation and death in from a few days to a few weeks. By beginning with very minute doses, however, the animals may be gradually habituated to intoxication by the dead bacilli and eventually withstand large doses. The same holds true of the various toxic substances, including tuberculin, which may be extracted from cultures. The proteins and alkaline extracts cause abscesses when given subcutaneously. The fever-producing substance which is present in the preparations mentioned below is one of the metabolic products of the bacillus, rather than a constituent of the bacterial cell (Koch). This substance is 100 times as toxic for tuberculous animals as for healthy and causes an increase in the eosinophiles of the blood. In addition to the fever-producing substance, Maragliano and others recognize as a constituent of the bacillus a heat susceptible "toxalbumin" (destroyed at 100° C.) which reduces temperature. Hammerschlag speaks of a toxin which in animals causes fatal convulsions. The toxic products of the

tubercle bacillus show their greatest toxicity when injected into the brain, and this method of injection has been suggested for the standardization of tuberculin.

Tuberculin. Of the toxic preparations of the bacillus the greatest interest attaches to tuberculin which Koch, in 1891, announced as an agent which could be used for the specific diagnosis of tuberculosis and which, when properly administered, had certain curative effects. Its preparation is simple. Cultures are allowed to grow for four weeks in peptone bouillon which contains 5 per cent. of glycerin. At the end of this time the organisms are killed by exposure to a temperature of 100° C. for one hour (Marx). The fluid is reduced to one-tenth its original volume by evaporation under a vacuum at a low temperature and the bacterial cells are eventually removed by filtration. The percentage of glycerin which is present in the final preparation acts as a preservative, but 0.5 per cent. carbolic acid may be added in addition. The active substance in tuberculin may be precipitated by 66 per cent. alcohol; its chemical nature remains unknown.

"TA," "TR" and "TO." In addition to the "old tuberculin," which has just been described, Koch has made several other preparations having similar properties, the use of which has been proposed for diagnostic and curative purposes and for convenience in carrying out the agglutination reaction. One of these, "TA," is an alkaline preparation which is made by extracting cultures with 1/10 normal sodium hydroxid solution. Its value as a diagnostic was equal to or exceeded that of tuberculin because of the longer duration of the reaction. In view of

the fact, however, that it contained undissolved cells, which caused the formation of abscesses at the point of injection, its use was not encouraged. For purposes of immunization Koch prepared a fluid which contained all the bacterial constituents and which at the same time is readily absorbed without abscess formation. For its preparation dried masses of the organism are ground up in an agate mortar; after suspension in distilled water and centrifugation, the emulsion consists of two layers. The overlying opalescent whitish fluid was designated as "TO" (*Tuberculin-Obers*). After removal of the fluid from the precipitate the latter was again dried and ground, suspended in water and centrifugated as before, and the process repeated until none of the sediment remained. The different fractions of fluid, except the "TO," were combined to constitute "TR" (*Tuberculin-Rest*), which is really an emulsion of minute fragments of cells. It is readily absorbed and does not cause the formation of abscesses. This is commonly called Koch's "new tuberculin." Still another preparation which Koch has recently devised for active immunization and for convenience in performing the agglutination test consists of dried and ground up bacilli which are suspended in equal parts of glycerin and water, *Neutuberculin* Koch (*Bazillenemusion*).

Other Tuberculins.

Preparations which in many respects are analogous to those of Koch have been made by different investigators; the tuberculocidin of Klebs, the tuberculins of de Schweinitz and Dorset and that of Denys, the two toxins of tuberculins of Maragliano, which he utilizes for the preparation of antitoxic serums, the oxytuberculin of Herschfelder, the

"TD" and the "TDR" of Behring and the tuberculoplasmin of Buchner. Marmorek claims to have obtained the true toxin of the tubercle bacillus by growing young, vigorous cultures on a complicated medium, denying that tuberculin represents the true toxin of the organism.

Standardization. Tuberculin can not be standardized with accuracy. Because of the extraordinary susceptibility of tuberculous animals to tuberculin, Koch decided to estimate its value by the quantity required to kill such animals. From 0.5 to 1 c.c. of tuberculin, when injected into a healthy guinea-pig, causes neither a local nor a general reaction, whereas from 0.1 to 0.15 c.c. kills a tuberculous guinea-pig in from twenty-four to forty-eight hours. For standardization von Lingelsheim recommends intracerebral injection into healthy guinea-pigs, because of the extreme toxicity of tuberculin when introduced into the central nervous system; only 1/180 as much tuberculin was required to cause death by intracerebral injections as compared with subcutaneous or intraperitoneal. Behring bases the value of tuberculin on its toxicity for healthy guinea-pigs and in his terms the expression "1 c.cm. = 1,000 M." means that one gram of the toxin is fatal to 1,000 grams of guinea-pig tissue. His "TD" has a value of 1,250 M., and "TDR," 12,500 M.

Dissemination. The tubercle bacillus undergoes no proliferation outside the body and its occurrence in nature depends on the distribution of the infected excretions, particularly the sputum, of man. Hence it is found most abundantly in the rooms and homes of patients and in tuberculous wards of hospitals. Reception of sputum on the handkerchief of the

patient, where it subsequently dries, and its discharge on the floor in public places, where it quickly becomes pulverized, as in street cars, are conditions which favor dissemination and the infection of others. In unconfined places which are exposed to the action of light and sun, as the streets and sidewalks, the danger is less on account of the shorter life of the organism under these conditions and the greater volume of surrounding air. The calculation of Heller that a tuberculous patient may excrete 7,200,000,000 of bacilli in a day suggests the number which may lurk in a single misplaced portion of sputum. Sputum which is kept moist is not a source of particular danger, inasmuch as ordinary currents of air do not dissipate it in the form of infected drops. Droplets of sputum which are expelled by coughing contribute greatly to the infected dust which surrounds a patient. **Dried Sputum**

Large quantities of bacilli are often excreted in the feces in intestinal tuberculosis and in the urine in genitourinary tuberculosis, or in general miliary tuberculosis with localization of the process in the urinary organs. The pus from tuberculous abscesses commonly is infectious.

Great interest attaches to the possibility of infection of man by the milk and meat of tuberculous cattle. Previous to 1901, through the work of Smith and others, the opinion had been gaining ground that the bacilli of human and bovine tuberculosis are not identical. It was not always possible to produce tuberculosis in cattle by feeding them or causing them to inhale tuberculous sputum or pure cultures which were highly infectious for other experiment animals, although bacilli of **Bovine and Human Tuberculosis.**

bovine origin invariably caused the disease in cattle when administered in a similar manner. It seemed then that the two bacilli are not identical in their pathogenic powers. Koch having performed such experiments without being able to infect cattle with bacilli of human origin expressed his belief that the converse also is true, i. e.,that the bovine bacillus is not pathogenic for man. Perhaps the strongest argument in favor of this view is the circumstance that primary tuberculosis of the inestines and mesenteric glands is very rare in children, who drink a good deal of milk, in spite of the great prevalence of tuberculous cows. Many protests followed the announcement of Koch's views, and in a short time a number of investigators showed, first, that it is possible in some cases to produce tuberculosis in cattle with tuberculous material from man, and, second, that infection of man with the bovine bacillus is possible. Unquestionable proof of the latter consists in the development of localized tuberculosis in those who have performed autopsies on tuberculous cattle (Ravanel and others). These occurrences, of course, do not prove the identity of the two organisms, for there is still abundant reason to believe that the two bacilli are most pathogenic for their respective, natural hosts, and much less pathogenic for the alternative hosts. Theobald Smith has pointed out that many experiments in which the pathogenicity of the human bacillus for cattle was investigated by the feeding of tuberculous sputum must be repeated, inasmuch as it was not determined in advance whether the organism contained in the sputum was of the human or bovine type. Naturally, absolute conclusions as to the patho-

genicity of the human bacillus for cattle could not be drawn with this fact undetermined. In some cases the combined sputum from many patients has been fed to cattle, and, since both human and bovine bacilli may have been administered, the results are valueless in relation to the point under discussion. In each instance the organism should be obtained in pure culture, its identity as a human or bovine bacillus determined and the experiment performed with such pure cultures. The following points serve to distinguish the bovine bacillus from the human: First, the bovine bacillus is shorter than the human; second, when first cultivated it grows feebly in media in which the human bacillus flourishes; third, it has a higher virulence for rabbits and guinea-pigs, and, fourth, it produces more extensive lesions in cattle. To these Smith has added a fifth point, which he has found to be distinctive in a large number of cultures. In bouillon which contains 5 per cent. of glycerin and which is 2 per cent. acid to phenol phthalein the bovine bacillus produces a neutral or faintly alkaline reaction in from three to several weeks, whereas the human bacillus, after causing temporary alkalinity, produces a terminal acidity of from 0.5 to 1.5 per cent. On the basis of this test and other points the bacilli of two cases of mesenteric tuberculosis in man were recognized as bovine in type. In view of the fact that infection of man with the bovine bacillus has been shown to be possible, we are still justified in considering the meat and especially the milk of tuberculous cattle as the probable sources of infection in a limited number of cases.

Differences in the Bacilli.

Comparatively few cases of undoubted congeni-

Congenital Tuberculosis.

tal tuberculosis have been observed, and in such cases the mothers are usually in an advanced stage of the disease. It is probable that the organisms reach the fetus following metastatic invasion of the placenta. In a number of cases in which the mother had advanced tuberculosis the organs and blood of the fetus (stillborn or dying soon after birth), contained very many bacilli, although histologic lesions had not as yet been produced. Warthin and Cowie suggest that the tissues of the fetus may possess considerable immunity in such cases. Baumgarten is a strong believer in the possibility that tubercle bacilli may pass to the fetus during pregnancy and, remaining latent in some of the tissues (lymph glands) for a long period, cause active tuberculosis later in life. Others who are less radical still admit that we should consider this as a possibility (Warthin and Cowie, Harbitz).

Infection Atria.

Pulmonary tuberculosis is by far the most common form of the disease in man, and without doubt this is due to inhalation of the dried and pulverized sputum of tuberculous patients. Drop infection may well occur in the case of those who are in intimate contact with the sick. In kissing, direct infection from mouth to mouth is a dangerous possibility.

The reason for the inception of pulmonary tuberculosis in the apex in so many cases is not clearly recognized, although it is often referred to the relative immobility of this tissue, which renders excretion more difficult and affords improper aëration. These conditions not only allow the organisms to accumulate and to proliferate, but the insufficient oxygenation probably causes a low tis-

sue resistance. The suggestion which has been made that apical tuberculosis is the result of extension of the disease from the cervical glands does not correspond with the condition seen in tuberculosis of adults in whom the cervical adenitis is commonly wanting.

The "anatomic tubercle" is a primary infection of the skin; lupus vulgaris, it is supposed, may be either a primary infection or secondary to tuberculosis in some other organ; ulcerative tuberculosis is usually a secondary lesion, often occurring by direct extension from tuberculous lymph glands. Tuberculosis of the nose is uncommon. Infection of the tonsils is not infrequent and probably is a common cause of secondary tuberculosis of the cervical lymph glands. Primary infection of the pharynx sometimes occurs and large, coarse granulations of this surface have been proved in some cases to be of a tuberculous nature. Tuberculosis of the pharynx and larynx, however, most often arises from infection with tuberculous sputum.

In the process of dust infection of the lungs, and also by other means, many organisms lodge on the mucous membranes of the nose, mouth, pharynx, trachea and larger bronchi, but usually without producing a tuberculous infection. On account of the movement of the ciliated epithelium, tortuosity of the nasal channels, excretion of the bacilli with mucus, the conditions at these points are not favorable for infection.

Tuberculous ulcers of the esophagus and stomach are very rare, as is primary tuberculosis of the intestines. Secondary tuberculosis of the intestines usually is caused by the infected sputum

which the patient swallows. Primary infection of the genital organs may arise from direct contact.

That tubercle bacilli have often been found on the hands and finger nails of the sick as well as on those who are intimately associated with them is a significant fact in relation to the possibility of infection by direct contact.

Metastases. From a given focus tubercle bacilli extend to other structures in several ways. On more or less theoretical grounds one speaks of "extension by growth" of the organism into contiguous tissues. The commonest method of extension, however, is that of metastasis by way of the lymph channels. When bacilli penetrate a surface, with or without the formation of a lesion at the point of entrance, as in the mouth cavity, intestinal canal, or bronchial surface, they are carried to the lymph glands of the region in which the tuberculous process is instituted. As in plague, the infection atrium at times is indicated by the set of glands which is involved. In certain localities the secondary invasion of other structures takes place directly without the intermediate involvement of lymph glands, as in tuberculous meningitis caused by extension from the middle ear, and tuberculous peritonitis or pericarditis by extension from the pleura. Very frequently tuberculosis of the lymph glands and other tissues heals spontaneously, as described below. In case such healing does not occur, metastases continue from one lymph gland to another and to new sets of glands until the larger lymph channels are reached, as a consequence of which extensive regional or general tuberculosis results. Accidental localization of a focus often causes a wide departure from the slow development just described. Not

infrequently tuberculosis in a lymph gland, which is contiguous to a large lymph channel, as the thoracic duct, invades the wall of the latter, the surface softens from caseation or liquefaction and the contents, impregnated with countless bacilli, are gradually thrown into the circulation. Miliary tuberculosis, first of the lungs and then of other tissues, through the arterial circulation, follows such an accident. A similar course with variations in localization, follows invasion of the walls of branches of the pulmonary artery or vein. Rupture of a focus into a bronchus is followed by regional or more extensive dissemination of the bacilli throughout the lungs by respiratory forces. A slower eccentric extension is seen, particularly in the lungs, in which smaller and larger areas of consolidation occur. By means of short lymphatic metastases into contiguous territory new foci are instituted, which eventually fuse with the original lesion. It is suggested and generally believed that bacilli may be carried longer or shorter distances by wandering phagocytic cells. When tuberculosis once involves a surface like that of the pleura, peritoneum, pericardium or pelvis of the kidney, the whole surface frequently becomes involved in thickly studded miliary tubercles. It is probable that a great deal of dissemination is accomplished by the movements of the fluids and the surfaces of these cavities. In other instances, as in the ureters, Fallopian tubes and spermatic cords, extension seems to occur in the submucous tissue by means of the lymphatics. The autopsy often discloses that tuberculosis which appeared to be "primary" in such organs as bones, suprarenal glands, and meninges was preceded by an old process in a

lymph gland from which metastases occurred to the tissues in question.

The Tubercle and Other Tissue Changes.

Certain anatomic conditions produced in tuberculosis which are associated with recovery from the disease, or the contrary, may be referred to. The tubercle, the histologic unit of the tuberculous process, is produced as follows, according to the interpretations of Baumgarten: When a bacillus reaches a lymph gland, for example, it multiplies slowly and, partly through its presence as a foreign body, but particularly through its toxic secretions, injures the surrounding connective tissue and endothelial cells to a certain degree. Under some circumstances, especially in the parenchymatous organs and lymph glands, this injury may be so great as to cause the death of the adjacent cells (focal necrosis). When it is of a lower order the connective tissue and endothelial cells respond to the stimulus by dividing mitotically and eventually accumulate in large numbers within a limited area surrounding the micro-organisms. Not only the endothelial cells of the lymph spaces, but also those of the adjacent blood vessels, take part in the proliferation, many of the vessels being obliterated in consequence. Not infrequently bacilli are ingested by the new cells, although the ability of the latter to destroy the organisms is not clearly established. Metchnikoff says that tubercle bacilli may remain intracellular for many months and, although not killed, the pathogenicity is decreased or destroyed. The new cells are of polygonal shape, are fairly rich in cytoplasm, contain large vesicular nuclei and are termed "epitheloid" cells.

Certain of the epitheloid cells, usually those in the center of the tubercle, where the bacilli are

most numerous, undergo atypical proliferation in that repeated nuclear division takes place without corresponding division of the cytoplasm. This process results in the formation of the multinuclear giant cell which is so characteristic of the well-developed tubercle, although not distinctive of the disease. According to Weigert, the failure of complete cell division is due to injury to the cytoplasm (partial necrosis) by the bacteria which the cell contains. Metchnikoff and others take a different view of the formation of giant cells, considering that they represent individual epitheloid cells which have fused to form a multinuclear mass. **Giant Cells.**

Still more remote from the center of the tubercle, that is, surrounding the epitheloid cells, wandering lymphoid and plasmal cells accumulate. Certain retrogressive changes, especially necrosis and caseation, characterize the further history of the tubercle, although these changes do not occur equally early nor with equal intensity in all cases. Necrosis begins in the center of the lesion, and the view is often expressed that the formation of the giant cell is the first indication of the retrogressive change. Cell degenerations, however, with karyorrhexis may occur before giant cells have formed. With the death of the central tissue there occurs sooner or later the death of many of the bacilli in this portion of the tubercle. The progressive formation of new tissue continues in the periphery as the degenerative changes take place toward the center; the tubercle enlarges, both epitheloid and the surrounding lymphoid cells increase correspondingly, and new giant cells form at the periphery of the necrotic center, only to be included in **Retrogressive Changes.**

Formation of Fibrous Tissue.

the degenerated area as the latter extends. In favorable cases, in which the virulence of the organism is low or the resistance of the individual strong, the tuberculous area is eventually surrounded by adult fibrous tissue which in a sense accomplishes the isolation of the infected area. Without question such a capsule of scar tissue is an obstacle to the extension of the tuberculous process, whether it surrounds a nodule in a lymph gland, a cold abscess or a tuberculous sinus. Further steps in the healing consist of caseation of the entire area, its partial or complete substitution by connective tissue (tuberculous scar), or partial impregnation with lime salts (calcification). Not infrequently the caseous portion of a nodule undergoes liquefaction, which some have referred to the action of proteolytic ferments. The contents of such foci finally become sterile. In the event that healing of this nature does not occur, the infection is transmitted to other organs as described above.

Caseation, Calcification and Liquefaction.

General and Secondary Disturbances.

The temperature, loss of weight, fever, increased cardiac action, and arteriosclerosis which are seen in tuberculosis indicate that the products of the bacillus have a profound effect on the functions of the body, and produce great disturbances in metabolism, although they seem to have no marked selective action for particular tissues. Many disturbances are secondary to changes produced in particular organs and are not referable to specific action of the toxins, such as those which are consequent on poor oxygenation in pulmonary tuberculosis, and the amyloid degeneration which follows prolonged suppurative tuberculosis.

Mixed infection, especially with the streptococcus, plays a very important part in the course of

Mixed Infections.

pulmonary tuberculosis, especially in the caseous and cavernous forms. Staphylococci, *B. pyocyaneus,* various diplococci, the pneumococcus, bacillus of Friedlander, diphtheria and pseudo-diphtheria bacilli, and the influenza bacillus are also found as secondary organisms in pulmonary tuberculosis. Some of them invade the surrounding healthy tissue, cause lobular consolidations, and in this way probably prepare a favorable soil for further extension of the tuberculous process. They doubtless hasten the liquefaction of caseated tissue, a step in the formation of abscesses. The high and irregular fever often seen in advanced tuberculosis is commonly septic in its cause, and a terminal streptococcus septicemia is not infrequent. It is evident that mixed infections may complicate attempts at serum therapy.

Principles of Prophylaxis.

The essential principles in the prevention of tuberculosis consist of, first, the early recognition of the disease, so that the patient may be properly treated and cured, if possible, with the result that a new center of contagion is avoided; second, the rendering of well-developed cases harmless by suitable isolation and proper disposal of infected excretions; third, the disinfection of the rooms, clothing, linen and surroundings of tuberculous patients. A fourth point, the prohibition of marriage among the tuberculous, is one of great consequence, although we have little ground to hope for its realization. A fifth point, not yet fully established, is the possibility of universal vaccination against the disease.

The collection of infected sputum in properly constructed water-proof paper boxes, which, with their contents, should be burned daily, is the safest

Disposal of Sputum.

method of disposing of this material, and the most effective means of preventing infection of the patient's surroundings. Metallic, glass or earthenware sputum-cups containing 5 per cent. carbolic acid are serviceable, but must be subjected to frequent cleansing. When sputum is collected on a handkerchief the latter should be boiled within twelve hours and not allowed to dry; that the hands of the patient are likely to be contaminated from the handkerchief is evident. In coughing, the handkerchief should be held to the mouth to catch droplets of sputum and saliva which are expelled. The ordinances and rules which prohibit expectoration in street cars and other public places should be enforced. When bacilli are discharged in the urine and feces or in the pus of tuberculous abscesses and sinuses, these secretions should be disinfected by suitable means (chlorid of lime). Healthy persons should come in contact with the tuberculous as little as possible, and the eating utensils of the latter should be used by no one else.

Disinfection.

The floor of a room which is inhabited by a tuberculous person should always be moistened before it is swept, in order to avoid stirring up the dust. After the death or removal of a patient, the entire surface of the room and all its contents should be thoroughly disinfected by appropriate means. The proper disinfection of the premises which were once occupied by a consumptive should be a legal requirement, just as similar procedures are demanded in the case of smallpox and some other contagious diseases.

The special hospital in which the indigent tuberculous may be properly cared for and isolated has

been a powerful factor in causing the decrease of tuberculosis which has been noted in many countries. The removal of a patient to such an institution means the elimination of an infected focus from the community.

Susceptibility. and Immunity.

Cold-blooded animals (fish, amphibians, reptiles), and most birds are not highly susceptible to tuberculosis, although special varieties of the bacillus cause the disease in certain of them under natural conditions. When tubercle bacilli are injected into the circulation of birds, they may remain in the blood and organs for months, producing little or no tissue change, although they retain their virulence for other animals (guinea-pigs). No animal exceeds the guinea-pig in its susceptibility to this disease. Goats and sheep are fairly resistant, and the same is probably true of the horse, although its artificial infection is not difficult. That different varieties of a species may vary in their susceptibility is illustrated by the field mouse, which is highly susceptible, and the white mouse, which is relatively immune. Although similar variations may exist among different races of men, they are not readily demonstrated. The high susceptibility which appears to exist among certain races, as the negro, may be explained in part by unhygienic methods of living, in which safeguards against infection are not taken.

Racial and Individual Variations.

The discovery of healed or healing tuberculous foci in 70 to 90 per cent. of all autopsies, in contrast to the 15 to 20 per cent. of deaths from tuberculosis, shows that susceptibility and immunity are subject to marked individual variations. The ability of an individual to overcome a tuberculous infection is referred in a vague way to an unusual

resistance on his part; his defensive powers are said to be strong. Although we remain to a large extent in the dark concerning these defensive powers, they seem to rest chiefly in the ability of the tissues to destroy the bacilli; that is, the resistance is antibacterial. Many bacilli may be destroyed by leucocytes or endothelial cells before they are able to cause tissue changes. It was stated previously that healing in many instances depends on isolation of the focus by epithelioid, lymphoid and plasma cells, and by connective tissue. On general grounds we may assume that a tissue reaction of this nature takes place with greater vigor and rapidity in a strong, healthy person than in one of lower vitality. Aside from the question of individual resistance, recovery or progressive infection may depend on the smaller or larger amount of bacilli which gained entrance to the body, as well as on their virulence. Experiments show that susceptible animals recover from minute doses, whereas they succumb to somewhat larger doses of bacilli.

Predisposing Influences.

Various external influences increase susceptibility and resistance. Tuberculosis is to no small degree a disease of the poor, who so frequently live in an undernourished condition, in crowded, dirty rooms, with little sunlight and fresh air. The disease is more common in the city than in the country, where an outdoor life is the rule. Alcoholism, diabetes, measles, scarlatina, whooping cough often, and influenza not infrequently, are precursors of tuberculosis. Conditions which favor anemia, as pulmonary stenosis (rare), predispose to pulmonary tuberculosis, whereas insufficiency of the left heart, accompanied by congestion of the

lungs, is not often associated with the disease, although it has no influence in preventing infection in other organs. Tuberculosis is more frequent during the first two or three years of life, when children are so commonly confined, than from the third to the fifteenth year, when they live in the open air so largely. From the fifteenth year to middle life or later the disease increases in frequency because of greater exposure to infection. Physicians who are familiar with tuberculosis in Scandinavian countries and in America comment on the extent to which tuberculosis develops among Scandinavians after they come to this country.

"Hereditary Tendency."

Nothing is commoner than the occurrence of several successive cases of phthisis in the members of a family, and the expression, heard on all sides, that "tuberculosis is in the family," indicates the general belief that a family tendency may be transmitted from generation to generation. During recent years, however, closer analysis of the conditions has led many to doubt the existence or, at any rate, the importance of family tendency or inherited predisposition, and to refer the frequent occurrence of tuberculosis in a family to the greater exposure to infection which is occasioned by close contact with a pre-existing case. Cornet, who has made a close statistical study of tuberculosis, discredits entirely the hypothesis of hereditary predisposition, and Cornet and Meyer refer to the "*habitus phthisicus,*" which we are disposed to look on as an objective evidence of hereditary tendency, as a result rather than a cause of pulmonary tuberculosis. It is fair to say that the development of tuberculosis in several members of a family is not *prima facie* evidence of the existence of a

family predisposition for the disease. Where there are tubercle bacilli there is likely to be tuberculosis, and the occurrence of the infection in one furnishes the prerequisite, that is, bacilli, for the development of the disease in other members of the family. It is probable that the verdict of family tendency has often been pronounced erroneously. At the present time, however, we may not be justified in considering the subject a closed chapter.

Concerning Acquired Immunity.

It is the commonly accepted opinion that recovery from tuberculosis does not confer immunity to subsequent attacks. Cornet and Meyer suggest as an explanation of this condition that the local lesion is so strictly isolated that a sufficient amount of toxin does not escape into the circulation to cause a general reaction, hence the formation of antitoxin or other antibodies is impossible. This explanation seems inadequate, however, when we remember the strong antitoxic immunity which develops in tetanus and diphtheria in spite of the localization of the bacteria. The results of artificial immunization, in which unlimited amounts of toxic material or bacilli may be injected without the formation of satisfactory antitoxins, seem to indicate that the toxic constituents of the tubercle bacillus lack the power of causing the formation of a strong antitoxin.

In opposition to the prevailing opinion, certain observers find ground for the belief that recovery from local tuberculosis of the lymph glands, skin or bones, actually does render the patients immune to pulmonary consumption (Maragliano and others). In early experiments Koch noted that when tubercle bacilli were injected subcutaneously into guinea-pigs which were suffering from general tu-

berculosis, the subcutaneous inoculation remained as a local infection and not infrequently healed after sloughing. The general infection would seem to have increased local resistance. Although other investigators failed to duplicate the observation of Koch, this result is said to have suggested to him the idea of active immunization as a cure for tuberculosis, a method subsequently practiced by treatment with the various tuberculins.

Active Immunization.

In the United States, Trudeau and de Schweinitz, and in Europe, Koch, Behring, Maragliano and Baumgarten, with their followers, have practiced assiduously the artificial immunization of animals with the tubercle bacillus or various preparations from the organism, with the hope of producing active immunity to the disease. Some of the procedures, especially those of Koch, have been transferred to man as curative measures. In addition to active immunization of man, Maragliano especially has prepared an antituberculosis serum, to which he assigns antitoxic and bactericidal properties, and which he and others claim to have used with good results in the treatment of tuberculosis. Marmorek also prepares an "antitoxic" serum.

It has been shown that active immunization may so increase the resistance of various domestic animals (guinea-pig, sheep, rabbit, dog, calf, cow, etc.), that they withstand doses of bacilli which are invariably fatal for control animals. When the bacterial cells are used for immunization it is customary to begin treatment either with killed bacilli, or with living cultures which are naturally of low virulence, or the virulence of which has been lost by prolonged artificial cultivation. Relatively

avirulent strains as those cultivated from fish, turtle or fowls, have been utilized for the first injections. As immunization progresses one of two processes may be followed: either the quantity injected may be increased gradually, as when killed or avirulent bacilli are used, or the immunization having been begun with avirulent living cultures those of higher virulence may be substituted later. In any case immunization is difficult and slow, and many animals may be lost from cachexia or from tuberculosis which develops from hasty progression in dosage. The subcutaneous injection of intact cells has the disadvantage that local abscesses frequently develop, and to avoid this the intravenous injection of smaller doses has been practiced in some instances. For active immunization the "new tuberculin" of Koch containing all the cellular constituents in a finely divided form has the advantages that it may be given subcutaneously without abscess formation and is absorbed with some rapidity. An animal or person immunized with TR is immune to all the constituents of the bacillus. The condition produced by active immunization is one of increased resistance rather than of absolute immunity; large doses of bacilli may cause infection. The nature of the new resistance is not satisfactorily established.

Tuberculin in Diagnosis.

Inasmuch as tuberculin is used not only for diagnosis but also for curative purposes in man (active immunization), and since the principles of action are similar in both instances, the use of tuberculin may be considered at this point. A healthy man is not susceptible to moderate doses, but a tuberculous man is even more susceptible to the toxin than the tuberculous guinea-pig,

since 0.001 c.c. often causes an intense reaction. E. Weigert classifies the disturbances which tuberculin may produce in the tuberculous as thermal, circulatory, respiratory, digestive, nervous and vasomotor, and secretory. Necrosis may be produced at the point of injection. In so far as the diagnostic use of tuberculin is concerned, we are interested chiefly in the thermal disturbances, which are accompanied by chills, malaise and muscular pains. Following injection of a suitable quantity, a period of incubation of from eight to fourteen hours follows, and at the end of this time the temperature rises progressively for two or more hours and may reach a maximum of from 40° to 41° C.; after remaining at this point for from two to six hours, it recedes rapidly. In addition to this general reaction, the toxin causes congestion, redness and swelling at the site of the tuberculous lesions, i. e., the foci become surrounded by an inflammatory reaction. This is seen most readily in the tubercles of lupus vulgaris, and in the lungs declares itself by an increase in râles and expectoration, caused by the exudation accompanying the inflammatory reaction.

For diagnostic purposes the technic of administration is as follows: It must first be assured that the patient has no continued fever by noting the temperature every two hours for several days. One milligram of tuberculin is injected subcutaneously, this amount being obtained by suitable dilution of the original solution. If no temperature is produced by this amount, 5 or 10 mg. may be given in a second injection after an interval of two or three days. When the quantity is determined which causes a rise in temperature of one-half

degree C. or more, the dose is to be repeated after the temperature produced by the first injection has subsided. Two positive reactions should be considered necessary for the diagnosis of tuberculosis. One who, after injection of 10 mg. on two different occasions, gives no reaction is to be considered free from the disease (Marx).

Limitations in Diagnostic Use of Tuberculin.

Experience has taught certain limitations to the diagnostic value of tuberculin: 1. The test can not be applied to febrile cases inasmuch as the pre-existing fever could not be separated from that which the tuberculin might produce. 2. Cases of advanced tuberculosis frequently fail to give the reaction. The tissues of such patients have become resistant to the poison. 3. It is said that tuberculin frequently causes a similar reaction in those suffering from leprosy, actinomycosis and syphilis. Cornet and Meyer suggest that the phenomenon as it occurs in leprosy and actinomycosis is to be considered in the nature of a "group reaction" in view of the close relationship of the tubercle bacillus to actinomyces and *Bacillus lepræ* It does not always occur in syphilis, and in positive cases a latent tuberculosis may be responsible for the reaction. By a number of writers the facts just stated are taken to indicate that the reaction is not of specific character; that it may often be obtained in the tuberculous by the injection of apparently indifferent substances as trypsin, peptone (albumose), sodium cinnimate and the "mycoproteins" of other bacteria provides additional support to this view. On the other hand, since relatively large amounts of these indifferent substances are required to produce the reaction, whereas minute amounts of tuberculin suffice, others

hold that the specificity of the latter substance may be maintained.

Early tuberculosis reacts to tuberculin in the most typical manner. On account of the fact that latent or healing cases may respond to the test, its positive outcome gives no indication of the seriousness of the patient's condition, which is a practical question of some importance.

Danger (?) in Use of Tuberculin.

The fear that tuberculin, in producing an inflammatory reaction around tuberculous areas, may cause a dissemination of the bacilli, has acted strongly in preventing the use of the toxin for both diagnostic and therapeutic purposes. On *a priori* grounds, such an event would seem to be a possibility, for, with the inflammation, the vessels surrounding the tubercles become congested, new leucocytes accumulate and there is an extravasation of fluid. Since during the subsidence of the inflammation a certain number of leucocytes may again leave the area and as the extravasated fluid returns to the circulation, bacilli may be carried to other tissues by them. Contrary to such reasoning, however, the observations of Koch and his followers in animal experiments and in the diagnostic and therapeutic use of tuberculin in man, lead them to say that tuberculin when properly administered never causes an aggravation or extension of the disease. Similar conclusions were reached by Trudeau, Baldwin and Kinghorn in animal experiments in which, "as in previous observations, a favorable absorptive influence was noted on the diseased focus." Bearing in mind the limitations mentioned above, and the possibility of the reaction being induced by leprosy, actinomycosis and syphilis (?), the statement of Osler

may be quoted that "in obscure internal lesions, in joint cases and in suspected tuberculosis of the kidneys the use of tuberculin gives most valuable information"

Tuberculin Therapy.

The original unfavorable results which were obtained in the therapeutic administration of tuberculin are referred by Koch, Petruschky and others to improper selection of cases. Those in a febrile condition and those in whom destruction of tissue is advanced are not suited for the treatment, and in them little or nothing is to be hoped from the administration of tuberculin. Its curative value is supposed to depend on the local inflammatory reaction which it causes around tuberculous foci, and perhaps also on the necrosis which Koch claims is caused in the tubercles themselves. It must be the object during the whole course of treatment to administer the toxin in such doses that a moderate or minimum local reaction occurs. Larger amounts which would cause febrile reactions and eventually render the patient resistant to tuberculin and thus preclude the local changes are to be avoided. It is customary to begin with 1/10 to 1/20 milligram and gradually to increase the amount injected. If fever is caused by a particular dose, larger amounts are not to be given until fever ceases to follow this dose. By the time a dosage of 50 milligrams is reached, which may require many months, the patient usually has lost the power of reacting and the injections are to be interrupted until he again becomes sensitive to the toxin (from three to six months), after which treatment should be resumed. Cure is recognized when the patient has lost permanently the power to react, his condition then being identical with that of the healthy man.

Numerous German writers on the basis of practical experience assign an unquestionable curative power to tuberculin when administered as described. Its use has not extended widely.

The principles on which the action of tuberculin depend are hypothetical. Marmorek says that the fever and local changes are due to a special toxin (the true toxin), which the bacillus secretes under the stimulation of the tuberculin. Ehrlich supposes that cells adjacent to the tubercles have been injured moderately by the tuberculin which is produced *in situ*, and that as a consequence of this injury such cells are particularly susceptible to the additional tuberculin which is injected, and react to the stimulus by proliferation (Marx). In accordance with this conception the fever also in some obscure way is related to the local reaction. Investigations are needed to clear up this point.

Treatment with TR and "New Tuberculin."

In active immunization with TR, in which the solid constituents of the bacilli are injected rather than the toxic tuberculin, the cure is supposed to depend on the development of immune bodies rather than on local tissue changes. Koch published favorable results from its use, but reports from other sources were less satisfactory. Koch's *Neutuberculin* (*Bazillenemulsion*) is used in a similar manner. Koch proposes to use the agglutinating power of the patient's serum as an index of the immunity caused by the injection. The formation of agglutinins perhaps indicates in a general way the ability of the patient to form antibodies, but from the well-known fact that the agglutinating power does not go hand in hand with the protective power of serum in relation to many infections, this method of estimating the degree

of immunity does not rest on a good basis. The agglutination reaction is carried on with the emulsion which is used for immunization. Treatment in man is begun by the injection of 0.0025 mg. of solid substance and the amount is increased rapidly every day or two until a reaction occurs with a temperature of from 1.5° to 2° C. After a pause of a week the injections are begun again and eventually a dose of 20 mg. may be given. During treatment the agglutinating power of the patient's serum is tested frequently, and if it is not sufficiently high intravenous injection of the fluid portion of the emulsion may be practiced. The agglutinating power may go as high as from 1 to 25 to 1 to 150, rarely 1 to 200 or 300.

With both TR and the last preparation animals may be successfully immunized against tuberculosis.

Serum of Maragliano.

Maragliano publishes the following conclusions. "1, that it is possible to produce a specific (serum) therapy for tuberculosis; 2, that it is possible to immunize the animal organism against tuberculosis as is done in other infectious diseases, and that there is good reason for hope for an antituberculosis vaccination for man." He recognizes bactericidal, antitoxic and agglutinating properties of the serum as normal defensive powers of the body, and says that these powers are increased as the result of immunization. The bactericidal power of the serum is determined by its ability to inhibit the growth of cultures of the tubercle bacillus; its antitoxic power by its ability to neutralize a test poison which is obtained from cultures by macerating them in hot water; and its agglutinating power is tested with the homogeneous cultures of

Courmont and Arloing. For the immunization of animals a soluble toxin prepared by the filtration of young cultures, and also the intracellular toxins which are extracted by water from killed virulent bacilli, are injected. By using both substances, antitoxic and other antibodies are said to be formed.

The unusual claim is made by Maragliano that his antituberculosis serum is effective in the treatment of human tuberculosis not only because of its own properties, but because it causes the tissues to form additional antibodies. It is difficult to take the latter claim seriously, since it is not in accord with the laws of anti-body formation as we understand them at the present time. However, favorable reports of the value of the serum have been published principally from Italian sources. It is claimed that the serum manifests its curative powers and causes an increase in specific antibodies when given *per os*.

Mixed Immunization and Vaccination.

Maragliano also advocates a method of mixed active and passive immunization in man, in which a cubic centimeter of serum is given subcutaneously every second day for twenty days; for a second period the same quantity of serum is given, but supplemented by increasing amounts of the toxic extract of bacilli; and for a third period the toxic extract is injected in increasing doses during from three to four months.

The same authority thinks it may be possible to vaccinate against tuberculosis by a single subcutaneous injection of a vaccine (killed bacilli?). He states that in both man and animals antibodies are formed in the serum following the vaccination, and that in animals their resistance to infections with

living bacilli is increased. Marmorek asserts that killed tubercle bacilli which have been treated with his antitoxic serum are readily absorbed from the subcutaneous tissue, and proposes the use of such bacilli as a vaccine.

Serum of Marmorek.

As stated above, Marmorek discredits tuberculin as the specific toxin of the bacillus. His "true" toxin is prepared by growing young and virulent bacilli ("primitive" bacilli) in a medium which contains leucotoxic serum, liver extract, glycerin and bouillon. The leucotoxic serum is prepared by immunizing calves with the leucocytes of guinea-pigs. Theoretical considerations which we need not detail suggested the use of this medium. The cultures are filtered after a few days of growth and the formation of tuberculin is avoided as much as possible. The immunization of horses with this filtered toxin yields the antitoxic serum of Marmorek. Conflicting reports concerning its value are published from French sources. Schwartz announces the complete cure of a case of tuberculosis of the larynx, and another of virulent tuberculosis of the conjunctiva and cornea by the exclusive use of Marmorek's serum.

Immunization by Milk.

Both Maragliano and Behring affirm that the immunizing substances are excreted in the milk of cows which have been strongly immunized against tuberculosis, and both have suggested that the use of such milk by infants may render them more resistant to tuberculosis.

The agglutination reaction has been suggested by Courmont and Arloing and others as a means of diagnosis in tuberculosis. Others who criticise this idea affirm that agglutinins are not developed sufficiently in tuberculosis to render the test of

value, and that the serum of normal man may be as highly agglutinating as that of the tuberculous. In view of the fact that the tubercle bacillus grows in coherent masses in ordinary cultures special manipulations are necessary to render it suitable for the agglutination reaction. Courmont and Arloing prepare a homogeneous culture by first growing the organism on a certain potato medium and then in glycerin bouillon, which is frequently shaken. The cells are said to be well isolated after this procedure. Koch uses his emulsion of powdered bacilli for the test. The serum of man or animals as a result of immunization may reach an agglutinating power of 1 to 2,000 exceptionally (Maragliano).

APPENDIX.

TUBERCULOSIS AND PSEUDOTUBERCULOSIS IN ANIMALS.

Bovine Tuberculosis.

Certain differences between the bacilli of human and bovine tuberculosis were mentioned in the preceding section. In cattle the disease shows a characteristic tendency to remain localized in one organ or group of organs over a long period. It is a nodular disease as in man, but differs from human tuberculosis in that nodules often grow to large size, may be imbedded in and sharply differentiated from surrounding healthy tissue, and not infrequently involve serous surfaces, forming large masses of firm sessile or pedunculated tumors. The nodules frequently are fibrous from the beginning, undergo early and extensive calcification and rarely soften. We are not to understand, however, that miliary tuberculosis does not occur in cattle. Although the process in the lungs is usually of a fibrous and large nodular nature, rapid dissemination with formation of many miliary tubercles may cause the picture of acute tuberculous consolidation in a certain number of cases. According to the statistics of Ostertag, based on 43,000 cases of bovine tuberculosis, localization is as follows: Lungs, 75 per cent.; pleura and peritoneum, 50 per cent.; peribronchial glands, 60 per cent.; spleen, 40 per cent. In more or less generalized cases the lungs are involved in 100 per cent. of the cases; serous membranes,

90 per cent.; liver, 85 per cent.; digestive tract, 60 per cent.; spleen, 50 per cent.; kidneys, 30 per cent.; mouth cavity, 5 per cent. In cows the uterus, in general infection, is involved in 65 per cent. of the cases, the udders in from 5 to 10 per cent., and the ovaries in 5 per cent. It seems that the lungs are the most common infection atrium, and transmission probably is accomplished chiefly through the secretions of the respiratory passages. In the udders the process may at first be one of miliary tuberculosis, but a large amount of fibrous tissue forms in time, many acini are transformed into retention cysts, in which tubercle bacilli, free or intracellular, may be present in large numbers.

Aside from anatomic changes and clinical symptoms, diagnosis depends on the tuberculin reaction, and, in relation to the udder, the demonstration of bacilli in the milk by staining methods or inoculation into guinea-pigs.

The tuberculin reaction in cattle is similar to that in man and is subject to the same general limitations, but is used extensively with the most satisfactory results. The complete elimination of tuberculosis from herds of cattle is possible, by using tuberculin as a diagnostic test, the slaughtering of infected animals, and the disinfection of stalls.

Tuberculosis among sheep and goats is rare. It occurs occasionally in the horse, hog and dog, and with more frequency in the cat.

Avian Tuberculosis.

A form of tuberculosis is very common in the chicken, and attacks also the pheasant, dove and turkey. The duck and goose are exempt from it. Although the organism resembles that of human tuberculosis in size, staining properties and other general characteristics, differentiation is accomplished by means of the following points: 1. The avian bacillus shows a greater tendency to pleomorphinism as shown by club-shaped forms, unstained vacuoles, "spore-like" bodies, and branching threads. 2. It has a greater affinity for aqueous anilin dyes. 3. Growth takes place in artificial media more rapidly and on solid surfaces is characterized by its moist appearance and mucus-like consistence in contrast to the dry, warty, brittle growth of the human bacillus. 4. The optimum temperature for growth (from 40° to 45° C.) is several degrees higher than that of the mammalian organism. 5. Its pathogenicity for guinea-pigs is less and for rabbits greater than that of the human and bovine bacilli. Their difference in pathogenicity is further shown by the difficulty which is met in trying to

infect fowls with the human bacillus. By varying the conditions of cultivation and by animal passage the two may be made to resemble each other very closely, although the permanent transformation of the human into the avian, or vice versa, has not been accomplished.

The disease attacks especially the intestines, mesentery and liver, in which are found hard, yellowish-white nodules, often rich in lime salts, and varying in size from that of a pea to that of a walnut. These conditions suggest the intestines as the infection atrium. The foci are rich in bacilli and histologically show the essential characteristics of tuberculosis.

Tuberculosis of Fish, Etc.

"*Bacillus tuberculosis piscium*" is the name given to an acid-fast organism resembling the tubercle bacillus which was cultivated from an inflammatory tumor in the abdominal wall of a carp. It grows well at low temperatures, the optimum being 25° C., is found in large numbers in the lesions within giant cells, and is distinctly pathogenic for frogs. Certain authors state that the human bacillus when inoculated into the frog undergoes changes in its cultural and pathogenic characteristics, eventually resembling the organism cultivated from fish.

Similar bacilli have been cultivated from a form of tuberculosis in the turtle (Friedman), and *Blindschleiche*—blind worm (Moeller).

OTHER ORGANISMS RESEMBLING THE TUBERCLE BACILLUS.

Certain other organisms of low pathogenicity resemble the tubercle bacillus in their acid-fast properties, their ability to grow in the form of branching threads, and to produce tubercular or nodular infections of a local nature in animals. They may be placed in a group which includes the tubercle bacillus.

Smegma Bacillus and the Bacillus of Lustgarten.

C. Fraenkel, also Neufeld, recognize in smegna two acid-fast bacilli, calling one "tuberculoid" because of its morphologic resemblance to the tubercle bacillus, and the other "diphtheroid" since it shows the pleomorphism of the diphtheria bacillus. One of these organisms may be identical with the "syphilis bacillus" (?) of Lustgarten. Smegma bacilli are most numerous beneath the prepuce in man and about the clitoris and vulva in women. Their chief significance lies in the danger that they may be mistaken for tubercle bacilli in suspected cases of genitourinary tuberculosis. Smegma bacilli may readily enter the urethra in women and be carried into the bladder during catheterization or cystoscopic examination. In man the danger of bacteriologic error may be elimin-

ated largely by cleansing the glans and carefully irrigating the urethra. Urine which is then passed is not likely to contain smegma bacilli (Young and Churchman).

Bacilli from Milk, Butter and Grass.

"Milk bacilli" and "butter bacilli" are acid-fast organisms resembling the tubercle bacillus morphologically. In injecting milk into guinea-pigs as a test for tuberculous contamination, Petri occasionally noted, as a consequence, a thick membranous growth which encased the liver and spleen and bound the coils of intestines together. The omentum was thickened, and this structure and the mesenteric lymph glands contained nodules. In pure culture the organism is pathogenic for guinea-pigs only when given in large doses, and may kill the animals in several weeks with the anatomic changes noted above. Its virulence is increased by the simultaneous injection of butter. It is not pathogenic for man (Herbert).

Moeller cultivated organisms resembling the tubercle bacillus from timothy (timothy bacillus), from manure, and a third (grass bacillus II) from the dust of a manger. The last is marked with great pleomorphism, thread formation and motility in young cultures.

The leprosy bacillus and the B. of Lustgarten which resemble the tubercle bacillus will be considered later.

PSEUDOTUBERCULOSIS IN ANIMALS.

Although some of the organisms described above are often called pseudotubercle bacilli, the term pseudotuberculosis is now applied somewhat specifically to a nodular disease occurring in rats, mice and sheep (and perhaps in other domesticated animals), and in which organisms differing from the tubercle bacillus in staining and culture properties, morphology and pathogenicity, are found. The clinical course and anatomic changes are similar in the three animals mentioned, although the organisms are different. The lymph glands near the infection atrium become enlarged chiefly by a cellular infiltrate rather than extensive proliferation of fibrous tissue. The nodules undergo a soft caseation very early and rarely show calcification. The infection finds its way to other sets of lymph glands and may become more or less generalized with the formation of smaller and larger sized nodules.

Rats and Mice.

Pseudotuberculosis of rodents, occurring spontaneously in rats, guinea-pigs, rabbits and cats, is caused by an organism of considerable pathogenicity, and may occur in epidemic form in laboratory animals. Chickens also may contract the disease. Intraperitoneal inoculations

in guinea-pigs are fatal in a few days. Spontaneous infection takes place through the intestinal tract, and regional organs show the principal changes. The liver and spleen contain many nodules which may be as large as a hazelnut, and which are frequently caseated in the center. The organism is called *Bacillus pseudotuberculosis rodentium* or *Streptobacillus pseudotuberculosis dor.*

The disease in mice is caused by a diphtheria-like organism called *Bacillus pseudotuberculosis murium* and is pathogenic especially for the gray mouse.

Sheep.

A similar infection in sheep is of more importance and occurs with some frequency. It is called pseudotuberculosis ovis, and the bacillus has a corresponding name. The organism is supposed to gain entrance through wounds in the feet and legs, following which the adjacent lymph glands become involved, and the infection may be transferred to the lungs and other organs through the lymphatic circulation. The lesions are nodular, of varying size, usually surrounded by a fibrous capsule, and are either semipurulent or undergo early caseation. They may be found in all the visceral organs.

An organism resembling that cultivated from the sheep has occasionally been found in nodular conditions in cattle.

II. LEPROSY.

Course of Extension.

Leprosy existed in Egypt in prehistoric times and extended to another land only when intercourse was established between the two countries. It reached Greece at about 345 B. C., Italy in the first century before Christ, and from the latter country extended to Germany, France and Spain. Crusaders returning from the Orient also brought back the disease in later times and eventually all Europe was infected. Leprosy is known to have existed in Great Britain in the tenth century, and from that country it was carried to Iceland and Greenland. From Germany it extended to the Scandinavian countries, and from the latter to Finland and Russia. It also reached Russia from the South and East, and in the South it was at

one time called the Crimean disease. The West Indies and South America probably were infected from Spain, and through these channels the disease was carried to the southern states. The leprosy of the western states seems to have been imported by Norwegian immigrants chiefly. In 1902 the United States leprosy commission found 278 cases in this country. One hundred and eighty-six of these individuals probably contracted the disease in this country, 120 were born in foreign countries and 145 were native born. The disease also extended around the globe in the opposite direction, reaching China, Japan and the East Indian islands from India. The Sandwich Islands became infected in the nineteenth century.

The contagiousness of the disease appears to have been recognized at a very early period. In 636 A. D. leprosy houses were instituted in Italy and other countries, and the practice of segregating lepers soon became general. The hospitals were called Lazarus houses in middle Europe and St. George houses in Scandinavian countries. Pipin and Charles the Great declared marriage between lepers illegal. The rapid disappearance of leprosy in middle Europe during the sixteenth century is ascribed largely to the segregation of the patients.

Bacillus of Leprosy.

In 1872 Hansen announced that small rods, sometimes intracellular and sometimes free, were to be found constantly in teased preparations of leprous tissue. These rods, leprosy bacilli, are now universally recognized as the cause of the disease, and in 1879 they were stained by Neisser and a year later by Hansen. The organism is non-

motile, has about the dimensions of the tubercle bacillus, the same staining reactions, and frequently shows a beaded appearance (degeneration forms (?)). It is said to take up dyes more readily than the tubercle bacillus, but the difference is not so great as to be distinctive. It stains by Gram's method.

Success in cultivating the bacillus has been reported a number of times, but the researches of others have failed to confirm these successes. Up to the present time it is probable that the organism has not been made to grow in artificial media. The resemblance of the bacillus to other acid-fast organisms, which are not pathogenic for animals, and the non-susceptibility of experiment animals to leprosy, are conditions which render very difficult the identification of a culture as that of the leprosy bacillus. Nicolli is said to have produced leprous nodules in monkeys by inoculating them with diseased tissue.

Dissemination.

So far as known the organism has no natural existence outside the human body, and it is disseminated only by the secretions of the diseased. It is discharged chiefly through the secretions of the nose and the upper respiratory passages, the surfaces of which are so commonly the seat of leprous ulcers, and also through ulcerating lesions of the skin. Expectoration, sneezing and coughing have approximately the same significance for the dissemination of leprosy bacilli as of tubercle bacilli. However, the organisms which are found in the sputum and nasal secretions appear to be largely degenerated, a condition which may lessen the infectiousness of these substances.

Transmission. The infectiousness of the leprosy bacillus is of a low character. "Epidemiologic experience teaches that infection occurs only through intimate and prolonged association with the diseased, in which doubtless uncleanliness plays a very important rôle" (Gotschlich). A leprous husband eventually infects his wife, and the children of lepers commonly develop the disease early in life. The high percentage of leprosy which is noted among the laundresses of infected localities indicates that the disease may also be transmitted by indirect contact. Gotschlich throws some doubt on the importance of dust infection since so many of the bacilli found in sputum appear to be degenerated. Nothing is known of the resistance and viability of the organism outside the body.

Infection Atria. On account of the early appearance and almost constant occurrence of leprous lesions in the nasal passages Stricker believes that the latter constitute the chief infection atrium; of this Hansen is not positive. Nasal ulcers may be present in latent or apparently healed cases. Kolle cites a case showing extensive involvement of the spleen and liver in which the intestinal tract was considered the infection atrium. In some instances in which the disease is first noted in the feet, the organisms are supposed to gain entrance with infected soil through abrasions in the skin. According to Cornil and Babes, infection may take place through the hair follicles and sebaceous glands. The theory of Jonathan Hutchinson that leprosy may be contracted through eating diseased fish, or that the latter in some way may render individuals susceptible to infection is not now credited. Hereditary acquisition of the disease is of doubtful occur-

rence, although the bacilli have been found in ova (Babès) and commonly are present in enormous numbers in the testicles. Hansen states, however, that he has never found them in the female generative organs.

Location of Bacilli.

The presence of large masses of bacilli in leprous tissues is a characteristic of the disease. To a large extent they are intracellular and they are often grouped in such a way as to resemble bundles of cigars. Hansen believes that the bacillus is essentially an intracellular parasite, and that it becomes extracellular only as a result of degeneration and disintegration of infected cells. Unna, on the other hand, considers their location in lymph spaces as most characteristic. They appear to be carried to distant parts through the lymphatics. Certain large vacuolated cells, the lepra cells of Virchow, the *globi* of Hansen, which are filled to bursting with the leprosy bacilli, are characteristic of the disease. Unna and others consider these bodies as zoöglear masses rather than as intracellular accumulations, and Kanthack interprets them as bacillary thrombi in the lymphatic vessels. The nodules, or lepromas, consist of granulation tissue, containing many round and epithelioid cells, lepra cells and occasional multinuclear giant cells. In cutaneous macules columns of round cells surround the blood vessels, there is some proliferation of epithelioid cells, but relatively few bacilli. The bacilli are most numerous in the nodular lesions. They are found in the Glissonian tissue of the liver, in the pulp and follicles of the spleen, in the glomeruli and interstitial tissue of the kidneys when these organs are involved, in the nerves in both the nodular and maculoanesthetic forms of the

disease, and in the vascular endothelium. They have been demonstrated often in the ganglionic cells of the posterior root ganglia. Their occurrence in these cells leads Metchnikoff to say that the latter have phagocytic properties.

Endotoxin (?)

In view of the chronic course of leprosy and the absence of signs of intoxication over considerable periods, it seems probable that the bacillus secretes little or no soluble toxin. From time to time, however, patients with tubercular leprosy develop fever, which may persist for weeks or months and eventually terminate in death. During such attacks the nodules not infrequently enlarge, become soft and later disappear. Lie conceives that such periods represent massive infection of the blood with the bacilli, and that at this time the latter undergo extensive disintegration and liberate endocellular toxins to which the toxic phenomena are due. It is a remarkable fact that intercurrent infections, as measles and smallpox, and the administration of potassium iodid, cause a similar enlargement, softening and final disappearance of leprous nodules, accompanied by marked degenerative changes in the bacilli. Hansen is of the opinion that the fever induced by these conditions has an actual curative effect, although its influence is not readily analyzed. He quotes the opinion of Danielssen that potassium iodid may be used to determine the cure of leprosy, which would be indicated by absence of a febrile reaction.

General confidence is not felt in the "leprolin" which Rost prepared from his cultures of the leprosy bacillus (?). His cultures are said to have been mixtures of micro-organisms.

Susceptibility and Means of Defense.

Because of the failure to cultivate the leprosy

bacillus, experimental work with the serum and cells of man and animals, by which conclusions as to the defensive powers of the body might be drawn, can not be carried out. It seems probable that all men are susceptible to leprosy under the proper conditions. Sauton states that children of from 4 to 5 years are particularly liable to infection. Other conditions which may increase susceptibility are of a conjectural nature. It is possible that leprosy predisposes to tuberculous infection (?).

The condition in leprosy seems to be that of an organism of low virulence against which the body possesses no decisive protective agency. The reactions for the most part are of a local nature, involving the proliferation of connective tissue and blood vessels, and the accumulation of lymphocytes. That phagocytosis by macrophages (lymphocytes, connective tissue, endothelial and ganglionic cells) is a factor which antagonizes the proliferation of the bacilli is suggested by the large number of bacilli which are found in these cells.

Prophylaxis.

The principles of prophylaxis may be illustrated by citing the practices in Norway. Originally all lepers were confined to institutions. At the present time, however, only indigent lepers and those who can not be suitably cared for at home are required to enter an asylum, where they live under the best hygienic conditions. Other patients are allowed to remain at home, with the understanding that they sleep alone and, if possible, have separate rooms, that their clothing, linen and eating utensils be used by no one else, and that proper precautions be taken in the washing of linen. Dressings and bandages must be burned. Under these regulations

the number of lepers in Norway has decreased from 2,870 in 1856 to 577 in 1900. Banishment to the Island of Molokai is practiced in the Sandwich Islands. Segregation of lepers should be brought about in this country.

Carasquilla attempted the production of an antileprosy serum by immunizing horses with the blood of leprous patients. Although a few favorable reports concerning its effects appeared it has not proved of value in the hands of others.

III. GLANDERS (FARCY).

Occurrence of the Disease.

Under natural conditions the horse is the chief sufferer from glanders or farcy, the former name being applied to the disease as it occurs in the nose, the latter when in the skin. These names are relics of the time when the two forms of the disease were not recognized as having a common etiology. In either locality the disease may be acute or chronic, and in the horse about 90 per cent. of the cases are chronic. The ass is occasionally infected, and in this animal, as well as in man, an acute general infection (bacillemia) frequently develops, in addition to the cutaneous and nasal lesions which characterize the disease. Fortunately, glanders in man is rare. Cows and rats are immune, or nearly so; the sheep, goat and dog have fairly high resistance, although they may be infected artificially; the dog and rabbit are moderately susceptible, and for the guinea-pig and members of the cat family (tiger, lion and leopard), the bacillus is very virulent. Infection of the last named animals has been noted in menageries as the result of feeding them with the meat of a horse which had died of glanders. The acute infection usually is fatal, and

complete recovery from the chronic form of the disease is infrequent. Something less than 50 per cent. of the chronic infections in man terminate in recovery.

Bacillus Mallei.

The specific microbe, *Bacillus mallei,* discovered in 1882 by Loeffler and Schütz, is an aërobic organism which has approximately the morphology and size of the tubercle bacillus, but lacks the acid-fast property of the latter. It stains with anilin dyes, especially carbol fuchsin, but not by Gram's method. With weak staining it shows a granular structure. It grows well on ordinary culture media, showing a characteristic appearance on potato. In unfavorable media it may produce threads, while under more favorable conditions coccus-like forms are seen. Marked involution forms occur on media containing 3 per cent. of sodium chlorid (Wherry). The optimum temperature for growth is from 30° to 40° C.

Resistance and Endotoxin.

The bacillus is only moderately susceptible to sunlight, by which it is killed in about twenty-four hours. It withstands freezing, lives for two or three weeks in a dried condition at room temperature, and is killed by a temperature of from 56° to 60° C. in from ten minutes to one and one-half hours, depending on the amount and character of the medium in which it lies. Its resistance to the ordinary disinfectants (corrosive sublimate, carbolic acid, etc.), is not high. Milk of lime and solutions of calcium chlorid are suitable for the disinfection of stalls. In culture media the organism secretes no soluble toxin, but it contains an endotoxin which probably is one of the constituents in the various preparations of mallein.

The method by which the mallein of Roux and

Preparation of Mallein.

Nocard is prepared is identical with that used in the preparation of the old tuberculin. A virulent strain of the glanders bacillus is allowed to grow for some time (from two weeks to two or three months) in bouillon which contains from 4 to 5 per cent. of glycerin, the culture is then sterilized by heat and the bacteria removed by filtration. The toxin is not destroyed by high temperature. Other preparations, also called mallein, are made by extracting ground-up bacilli with a solution of glycerin and water (Helman, Kalning), or with water alone (Kalning and others); by killing a liquid culture of the bacillus (Bromberg); or by precipitating bouillon filtrates with absolute alcohol (de Schweinitz and Kilbourne), or with ammonium sulphate or magnesium sulphate. The dry powders "morvin" and "dried mallein" are prepared by one or another of these precipitation methods.

Distribution of Bacilli and Infection Atria.

Glanders bacilli are found only in the tissues and secretions of diseased animals, and the nasal discharges of the latter are the chief means of contaminating feed, water and stables through which the disease usually is carried to other animals. The glanders bacillus does not readily penetrate the intact skin and mucous membranes, although occasionally it may gain entrance through the hair follicles or sweat ducts. In the presence of even slight defects in these surfaces, as those caused in the mouth or nostrils of horses by hay or other food, infection readily occurs. According to Nocard, invasion takes place commonly through the gastrointestinal tract following the ingestion of infected feed or water. Although involvement of the intestines and adjacent tissues frequently results,

the organisms may become generalized, causing the disease in the nose, skin or other organs, without the establishment of foci in the intestines.

In man infection occurs chiefly through abrasions in the skin, and perhaps also through the nose, to which the bacilli have been carried by soiled fingers or other means. Several cases of acute glanders, ending fatally, have occurred in laboratory workers as the result of accidental inoculation. There appears to be little danger to man in eating the meat of horses in which the disease was localized, provided the meat has been well cooked. Such meat was fed to soldiers in one instance with no ill results.

Tissue Reactions.

Variations in the course of the disease and in the intensity of the pathologic changes in different cases probably depend on variations in the resistance of the host and in the virulence of the parasite. In acute general infections in man, following an incubation period of from two to five days, during which the point of inoculation becomes violently inflamed, a severe febrile condition develops, which is accompanied by general pains, swollen joints, a macular eruption, and often muscular and subcutaneous abscesses. In a short time nodules and indurated cords, made up of a leucocytic exudate, edematous fluid and proliferating connective tissue cells, form in the subcutaneous lymphatic channels, and mark the progress of the infection toward the lymph glands. The nodules, and also the cords, commonly undergo softening, and abscesses form and rupture through the skin. Nodules similar to those in the skin develop in various organs of the body; in the nose they break down and constitute ulcers. In chronic infections the

lesions are of the same nature, although they evolve more slowly and tend to remain limited to particular regions. Nasal, pharyngeal, tracheal or pulmonary glanders are forms of the disease which are encountered in the horse. Connective tissue development is more marked in chronic than in acute glanders, although the peculiar liquefaction, suppuration and ulceration of the lesions occur in the former as well as in the latter. Moderate leucocytosis is found in the blood (12000-14000).

Protective Processes.

The nature of the pathologic changes found in glanders, the frequent chronic and the progressive course of the disease, and the fact that infection does not confer distinct immunity, are conditions which ally glanders very closely to tuberculosis, pseudo-tuberculosis and leprosy. The essential lesion is the "infectious granuloma," and it is probable that the new connective tissue which is formed is to no small extent a factor in limiting the extension of the infection. Nodules of glanders frequently are isolated by the surrounding reaction, the centers caseate and the contents eventually are discharged through the skin; cicatrization and healing in many lesions follow evacuation. Phagocytosis of the bacilli by the epithelioid cells and leucocytes in the nodules is said to be rather extensive. According to Nocard, there is no such thing as an acquired immunity to glanders; chronic glanders may at any time become acute. The cause of the natural immunity of cattle and some other animals seems not to have been determined.

Serum Therapy and Use of Mallein.

Treatment of glanders with immune serums has not been successful. Such treatment has been attempted with serum prepared by immunization with mallein (Semmer), and with the serum of

diseased animals (Hell and Toeper). The value of mallein in the diagnosis of glanders or farcy is similar to that of tuberculin in tuberculosis. Although it causes a rise in the temperature of normal animals when given in considerable doses, the reaction produced in infected animals is so much more intense, and occurs with such smaller doses, that it is generally considered as specific in nature. Some doubt, however, has been thrown on the specificity of the reaction from the facts reported by various observers that toxic substances from other organisms, as tuberculin and preparations from the pneumobacillus of Friedlander, *Bacillus pyocyaneus,* etc., cause similar phenomena in animals suffering from glanders. Wladimiroff asserts, however, that the reactions caused by these substances differ from that of mallein.

For diagnosis a dose must be used which causes no reaction in a normal animal, and this varies with different preparations. The typical reaction has two essential components: 1, A rise in temperature which begins in from six to twelve hours after the injection, reaches its maximum (from 40° to 42° C.) in from six to eight hours later, where it remains for a few hours, then gradually sinks, only to recur on the second day; 2, an edematous and inflammatory tumor at the point of injection, which begins in from six to eight hours, and runs its course in from three to eight days, ending in resorption (Wladimiroff). Veterinarians generally agree that mallein is a valuable diagnostic agent. Mallein also has been used in the treatment of glanders, but with rather doubtful results.

Bacteriologic diagnosis is accomplished by cultivating the bacilli from the abscess or secretions and

testing the virulence of the culture by animal experiments (guinea-pig).

Agglutination. Normal horse serum agglutinates the glanders bacillus in dilutions of from 1/200 to 1/700, that of the diseased animal in a strength of from 1/1600 to 1/2000. In some instances, however, infection causes no increase in the agglutinating power of the serum. Agglutinins are said to be formed more readily in man than in animals.

IV. RHINOSCLEROMA.

(See page 402.)

V. ACTINOMYCOSIS.

Actinomycosis is a chronic infectious disease of man and animals, the lesions of which present, characteristically, a central mass of purulent and necrotic material containing colonies of "ray fungi," about or through which is disposed an abundant growth of granulation or fibrous tissue. In young or rapidly progressing lesions the amount of purulent material is large, while in older lesions well formed connective tissue is more conspicuous. The disease prevails especially among cattle, although it is met occasionally in the horse, hog, sheep, dog, cat and other animals; man is infected not infrequently.

Although fungous threads had been found in diseases resembling actinomycosis in 1845 and later, Bollinger, in 1877, gave the first accurate description of the disease in cattle, and in 1878 J. Israel described it as a new disease in man. A short time later Ponfick demonstrated the identity of bovine and human actinomycosis.

The specific organism, *Actinomyces bovis et*

hominis, on culture media consists of a mass of delicate threads which exhibit "true branching" and which, to a certain extent, segment to form "spores." The radially arranged groups of cells which occur as macroscopic granules in the pus of the actinomycotic abscesses, and which give to the organism the name of the "ray fungus," are essentially a manifestation of parasitic existence, although colonies developing on media which contain serum or ascitic fluid may show a degree of "club" formation (Wright). Each granule represents a colony of organisms the members of which possess club-shaped extremities, and in the center of the mass and extending from it are many of the delicate threads found in cultures of the organism. It grows on various culture media, often as a mold, and stains by Gram's method. **The Fungus.**

The actinomyces is an organism of considerable resistance. Cultures remain alive for one year or more when in a dried condition and the spores in one instance germinated after having been preserved for six years. A temperature of 80° C. for fifteen minutes kills the spores (Bérard and Nicolas). When suspended in bouillon, spores are killed in fifteen hours by direct sunlight, but when thoroughly dried, approximately ten days' exposure produced no injury. **Resistance.**

Attempts to place the actinomyces in a botanic system have resulted in many differences of opinion. By some investigators they are considered as an independent family midway between the hyphomycetes and the schizomycetes (bacteria), others place them under the hyphomycetes in the group

of the streptothrix, while still others consider them as pleomorphous bacteria, placing them in the group cladothrix. Petruschky recognizes actinomyces, streptothrix, cladothrix and leptothrix as genera in the family trichomyces, the latter belonging to the order hyphomyces. Biological variations which have been encountered have led to the recognition of several species of actinomyces, among which are a number of non-pathogenic forms. Wright limits the term actinomyces to those strains which produce colonies of club-shaped organisms in animal tissues.

Artificial Infection.

Many attempts have been made to transmit actinomycosis to animals by inoculating them with the diseased tissues of animals and man, and with pure cultures obtained from these tissues. Although a number of experimenters have reported positive results, the attempts usually have been fruitless. Probably Wright has been more successful than others in producing actinomycotic lesions in rabbits and guinea-pigs by the inoculation of pure cultures. Colonies of club-shaped organisms developed with considerable uniformity. In many instances the infection remains localized, not causing the progressive and destructive changes which actinomycosis produces when it occurs naturally.

Transmission and Infection Atria.

The organism has been found on grains, straws and other kinds of feed, with which it may be implanted in the soft parts of the mouth (gums, tongue), or in carious teeth. Transmission to man by eating the meat of actinomycotic cattle has not been noted. In man the disease is primary in the internal organs (lungs, intestines, liver, brain, etc.) in a large percentage of the cases, whereas "lumpy jaw" is rare. The disease extends locally

by the gradual involvement of adjacent tissues, which in time become occupied by sinuses, abscesses and masses of connective tissue. Numerous "spores" and bacillus-like cells, having their source in the fungous threads, abound in the vicinity of a colony. The occurrence of such forms in leucocytes and other large mononuclear cells has led some to the view that the micro-organisms may be carried to neighboring tissues or to distant parts as cell inclusions. In cattle the disease usually is more chronic than in man, more fibrous tissue is formed and metastases in internal organs are less frequent. In man the lesions are more purulent in character, large abscesses sometimes form as in the liver, and metastases in visceral organs are more common. Cases of general actinomycosis are occasionally met with in both cattle and man.

Prophylaxis.

Little can be said in the way of prophylaxis against actinomycosis. Knowing the part that infected grains, straws, etc., play in instituting infection, the practice of biting or chewing grains or of using straws as toothpicks, evidently is one which affords opportunity for infection. The presence of carious teeth has often been suggested as a predisposing condition for infection.

Immunity and Susceptibility.

Practically nothing is known concerning the degree to which susceptibility to actinomycosis prevails, and the question of immunity to the disease remains unexplored. The inability to reproduce the infection in animals at will renders a satisfactory study of these questions very difficult. The presence of large numbers of polymorphonuclear leucocytes in the vicinity of the organisms suggests, but does not prove, that they may have some

influence in combating the infection. Surely the abundant mass of connective tissue which develops about the abscesses and sinuses aids in confining the process to a definite region.

That the iodid of potassium has a curative influence on some cases of actinomycosis seems to have been well demonstrated. The principles by which it produces its effects are unknown.

VI. MADURA FOOT.

Mycetoma. Mycetoma, or Madura foot, resembles actinomycosis in the formation of abscesses, sinuses and granulation tissue, but it shows a peculiar predilection for the foot, which probably is explained by the greater exposure of this part to infection. This disease differs from actinomycosis in that the course is more chronic and it is never accompanied by generalized infection. The bones are not involved so frequently as in actinomycosis. Granules similar to those of actinomycosis are found in the cells, which, however, do not assume the pronounced club shape seen in colonies of the ray fungus.

Two varieties of the disease are known, one in which the granules are brown or black, and another in which they are white or yellowish; the latter is encountered much more frequently than the former.

Pure cultures of the organism, which is called *Streptothrix maduræ* (Vincent), were first obtained by Vincent in 1894, and have been studied by a number of observers since that time. It bears a close resemblance to the actinomyces and by some is considered a variety of this organism. Differences between the black and white varieties are not

clearly set forth. The disease occurs in southern Asiatic countries, in northern Africa, and in the United States (rare).

VII. INFECTIONS BY STREPTOTHRIX, CLADOTHRIX AND LEPTOTHRIX.

Streptothrix Infections.

Cultures of streptothrix, differing from the actinomyces, have been obtained from the lungs in a number of instances and in various countries. They have been found in such lesions as bronchopneumonia, or more extensive consolidation of the lungs, and in cases of empyema. In other instances organisms which have been classed, some as streptothrix, others as cladothrix, have been cultivated from processes which resembled actinomycosis.

Nocard considers a streptothrix as the cause of *farcin du bœuf* (farcy of cattle), a disease encountered especially in the countries of southern Europe, and similar organisms have been cultivated from suppurating or granulomatous foci in other animals.

Leptothrix buccalis, a thread-like organism which does not form branches and, hence, is not an actinomyces nor a streptothrix, is frequently found as a saprophytic organism in the mouth cavity, and a similar fungus, *Leptothrix vaginalis,* has been encountered in the vagina. Although organisms of this type are relatively harmless, they have occasionally been found in diseased conditions of the tonsils and pharynx.

VIII. OIDIOMYCOSIS.

In 1894 Gilchrist described a skin disease, which has since been known as blastomycetic dermatitis, or blastomycosis or oidiomycosis of the skin. From a second case he cultivated a fungus which at first

"Blastomycetic" Dermatitis.

he was inclined to consider as an oidium, but later called a blastomyces. Since that time many similar cases, especially in Chicago and the adjacent territory, have been discovered and reported by Wells, Hektoen, Hessler, Hyde and Montgomery, Ricketts and others. In many instances the specific fungi have been cultivated.

Systemic Oidiomycosis.

Further investigations by Rixford and Gilchrist, Busse, Ophüls and Moffit, Hyde and Montgomery and others have brought to light the existence of systemic infections by fungi which resemble closely those found in blastomycetic dermatitis, and, in fact, cases in which the infection primarily was limited to the skin have gone on to generalized infection. The *Saccharomycosis hominis* of Busse and Curtis, blastomycetic dermatitis, generalized blastomycosis, and about a dozen cases in California, which at one time were considered to be of protozoön etiology (Rixford and Gilchrist), are closely related or identical processes which have as their cause a group of fungi, the individual strains of which may show considerable differences.

Nature of Fungi.

In those cases usually described as blastomycetic dermatitis or systematic blastomycosis, the fungus proliferates in the tissues by budding, and is found chiefly in the intraepithelial and subcutaneous abscesses, and in the granulation tissue and nodules of internal organs. Its appearance in culture media and its biologic properties are subject to considerable variations, at one time growing as a mold, at another time more like the typical oidium, and again resembling some form of yeast. Ricketts considers that the genus oidium is sufficiently broad to include all the types which have been described, and

that blastomyces is too narrow. The organisms which have been cultivated from the cases in California grow as molds, and they differ from those described by Gilchrist, Hektoen, Ricketts and others in that they form endospores and apparently do not bud in the tissues of the host (Ophüls, Wolbach). Ophüls calls this parasite *Oidium coccidioides,* agreeing with Ricketts as to the generic character of the group.

Histology.

The skin infection usually appears as a coarse warty and ulcerative lesion, in which the large papillæ and cutaneous areola are beset with minute abscesses; the process extends gradually and eventually may involve large areas. Histologically, the tissue shows an enormous epithelial hyperplasia with intraepithelial abscesses, and a richly cellular, granulomatous condition of the subepithelial tissue, in which giant cells and small abscesses are found. When the disease is systemic, various internal organs, especially the lungs, spleen and kidneys, are the seats of abscesses and nodules which contain the parasites in immense numbers. The lungs show lobular or more extensive consolidation.

Infection Atria.

The skin infection occasionally follows slight traumatism, while in other instances no predisposing condition is known by the patient. The occurrence of cutaneous lesions in crops has been noted, and suggests that in some instances they may originate as embolic foci from a pulmonary lesion which later heals or becomes latent. In the systemic infection the primary lesion appears to be in the lungs in most cases, from which the blood and other organs, including the skin, may be invaded. Pulmonary oidiomycosis simulates pul-

monary tuberculosis. In extensive involvement of the lungs the organisms may be demonstrated in the sputum. At present nothing is known concerning immunity to these infections.

Thrush.

Ophüls very properly suggests that thrush should be considered as one form of oidiomycosis. Thrush is of particular interest because of the early date at which its parasitic nature was recognized. Langenbeck and Berg, in 1839 and 1841, are cited as the discoverers of the fungus, and they reproduced the disease by inoculations with fragments of the membrane. The parasite was studied a little later by Gruby, Robin and others, and the latter gave it the name of *Oidium albicans*. Grawitz obtained it in pure culture in 1877 and demonstrated its pathogenicity for dogs and rabbits.

Cultures of the organism show differences in size, morphology, chemical activities and methods of proliferation, although the variations are hardly so wide as those found among the fungi cultivated from cases of "blastomycosis."

Systemic Infection.

Although thrush usually is considered a rather harmless affection, Virchow long ago showed that its filaments may penetrate the submucous tissues and even the lumens of blood vessels. In rare instances systemic infection, with abscesses in the brain, kidney and spleen or with nodules in the lungs, has been noted; in these cases the conditions resemble those found in systemic "blastomycosis."

The healthy person has little or no susceptibility to thrush, although a few cases of infection have been noted in individuals who were otherwise nor-

mal. Customarily it attacks only those who are in a low state of vitality, as poorly nourished children or those in advanced age, or those whose resistance is much lowered by some other disease (typhoid, diabetes, etc.).

Phagocytosis and Immunity.

Phagocytosis of yeast and oidium-like cells takes place when they are placed in the abdominal cavity of experiment animals (guinea-pigs). A number of leucocytes may fuse to form a plasmodial mass around one or more of the parasitic cells. Roger and Noisette caused an increase in the resistance of rabbits to thrush infection by the intravenous injection of small doses of the parasite. According to Noisette, an immune serum agglutinates only the strain used in the immunization.

Infections of other animals (horses, cattle) by oidium-like organisms, the trichophyton and other fungi which cause superficial diseases in the skin of man, and other fungi (aspergillus, mucor), which occasionally are pathogenic for man, will not be discussed.

GROUP V.

PROTOZOON INFECTIONS.

I. MALARIA.

Etiology. The etiology of malaria, which for long was supposed to be associated with impure and swampy atmospheres (malaria is from mal' aria, Italian meaning bad air), remained unknown until 1880, when Laveran discovered ameboid, half-moon shaped and flagellated forms of a parasite in the blood of the patients. In following years Golgi, Grassi, Marchiafava and Celli and many others took prominent parts in working out the different forms of parasites, their sexual characters and their relation to the different types of malaria.

Ross and MacCallum. The conception that mosquitoes may be influential in transmitting malaria is a very old one and its origin is unknown. In 1894 Manson suggested that the malarial organism may utilize the mosquito as an intermediate host where, after undergoing further development, it again becomes infectious for man. He was inclined to think that the flagella are reproductive forms, which are essential for an *extra corpus* life of the parasite. The proof of this came from MacCallum in 1897, who showed that the flagellated forms are really spermatozoites, the function of which is to impregnate female cells of the parasite. This was observed first in relation to halteridium, one of the organisms of avian malaria, and later in relation to the parasites of human malaria.

In the same year Ross found the pigmented, half-moon shaped parasites of æstivo-autumnal

fever in the stomach of the anopheles mosquito. Through the work of Ross and others it is now established that the malarial parasite undergoes further development, a sexual cycle, in anopheles, and that man is inoculated only by the bites of such infected insects. From the standpoint of the zoölogist, man is an intermediate host for the parasite, since the latter undergoes its higher development only after it reaches the mosquito.

Species of Plasmodium.

The malarial parasites of man belong to the class of Sporozoa; order, Coccidiomorpha; family, Hemosporidia; genus, Plasmodium. The following are the names given to the three species: 1. Plasmodium præcox (parasite of æstivo-autumnal fever); 2. Plasmodium vivax (of tertian fever); 3. Plasmodium malariæ (of quartan fever).

Tertian Fever.

When the blood of one suffering from tertian fever is examined at the end of the febrile paroxysm, or at the beginning of the afebrile stage, the parasites are found within the erythrocytes as pale, rather clear bodies, about one-fifth the diameter of the corpuscle, and in fresh specimens showing an active ameboid movement. They are very difficult to recognize in unstained specimens. They increase in size gradually, and after eighteen hours, when they begin to acquire pigment, they are recognized more easily. After twenty-four hours the pigment has increased markedly and the erythrocytes are swollen and pale. In stained preparations the periphery of the parasite stains more deeply than the center and gives it a pronounced ring form. At the end of thirty-six hours they have increased noticeably in size and their ameboid motion is less. Shortly before the next attack—i. e., forty-six to forty-eight hours after the preceding one—

Segmentation of Asexual Cells.

the pigment assembles into one or two groups in the center of the parasite and clear hyaline points begin to appear. These are the young endocellular parasites which are formed by division of the nucleus of the mother cell. They gradually increase in size and number, and as the red corpuscles disintegrate they are discharged, from fifteen to twenty-five in number, as young parasites. This completes the cycle, an asexual cycle, which has lasted forty-eight hours, and the young forms then begin a new cycle after penetrating other red corpuscles. The mother cell is called the sporocyte and its offspring are merozoites, and the process of division schizogony.

Sexual Cells.

In addition to the asexual cell just described, two sexual cells, a male and a female, grow to adult size in the erythrocytes, acquire pigment and eventually become free. They differ from the asexual cell in that the pigment continues to be uniformly distributed, and neither gives rise to young parasites by division. The male cell (microgametocyte, 8-9 microns) has a clear protoplasm and is smaller than the female (macrogamete, 10-14 microns); the female has a granular protoplasm. There are many more male than female cells. They undergo no further development in the body of man, and in order that the sexual process be completed the two cells must first gain entrance into the stomach of the female anopheles mosquito.

Impregnation.

A further step in the sexual process may be seen in drop preparations of the blood, although this step does not occur in the human body. Ten to twenty minutes after such a preparation has been made the male cells, after a period of agitation,

discharge from four to eight long, thin flagella (microgametes or spermatozoa), which thrash about violently and eventually come in contact with a female cell, which they enter and become unrecognizable.

Life in the Mosquito.

This same process is instituted and completed (sporogony) in the stomach of the mosquito, the penetration of the female cell by the spermatozoon resulting in the impregnation of the former. Following impregnation, the female cell gradually assumes a worm-like or sickle shape (ookinet), penetrates the wall of the stomach and becomes encapsulated (oocyst). Forty-eight hours after the mosquito has sucked malarial blood all the female cells have reached this stage and no more free parasites are found in the stomach.

Formation of Sporozoites.

About five days after the blood was taken the oocyst has increased in size about six times and has formed within itself a number of small spheres, which are called daughter cysts or sporoblasts. The latter soon acquire a finely striated appearance, which is due to the formation of hundreds of "germinal rods" or sickle-like bodies (sporozoites). The latter are nothing less than young malarial parasites, which are thrown into the body cavity by the rupture of the oocyst, and are carried to the salivary glands of the mosquito by the lymphatic circulation. If the mosquito has been kept at a temperature of 24° to 30° C. these sickle forms first appear in the salivary gland after eight to ten days. Such are the cells which are inoculated into man by the bite of the mosquito. The changes which they undergo before they appear as clear oval bodies in the erythrocytes are unknown.

Parasite of Quartan Fever.

The asexual cycle of the quartan parasite is identical with that of the tertian, with the exception that seventy-two hours are required for its completion. It contains more pigment, and when division takes place eight, or at most fourteen, young parasites are formed, in contrast to the fifteen to twenty-five of the tertian parasites. The erythrocytes do not become large and pale (Ruge). The sexual cells practically are indistinguishable from those of the tertian parasite, although they are, on the whole, slightly smaller. The sexual cycle also is completed only in the body of the female anopheles mosquito, and is identical with that of the tertian parasite.

Parasite of Aestivo-Autumnal Fever.

The parasite of æstivo-autumnal fever is one-half to two-thirds the size of the tertian parasite, a difference which is constant in the various stages of development of the asexual cell. It divides eventually into eight to twenty-five young parasites, the cycle occupying from twenty-four to forty-eight hours.

"Half-moon" Cells.

Here, as in quartan fever, the erythrocytes do not become swollen and pale, but even appear darker in color, because of some shrinking (Ruge). The sexual cells in æstivo-autumnal fever are characteristic. Whereas they at first do not differ in shape from the asexual cells, as they grow older they gradually assume the shape of a half moon in one edge of the erythrocyte, reaching a length equal to one and one-half diameters of the red cell. At this time a fine line drawn across the concavity of the parasite represents the margin of the erythrocyte. This form is only temporary, however; they subsequently assume first a spindle and then a spherical form. As in the other parasites, the

male cell is rather clear and the female granular. When mounted in a hanging drop the male cell liberates flagella, which penetrate the female cell. This does not occur in the human body. In this respect, and also in the completion of the sexual cycle in the body of the mosquito, they resemble the other two parasites.

The parasites of tertian and quartan fevers undergo division while they are in the circulating blood, and when peripheral blood is examined at the end of the afebrile stage the young cells may be found extracellular. This is not the case, however, in the æstivo-autumnal fever. In this instance, for unknown reasons, the adult cells withdraw to the internal organs, especially the spleen, bone-marrow and brain, where division takes place in the minute vessels. Hence if the peripheral blood is examined preceding and during the febrile stage few or no dividing cells or young parasites are seen.

Incubation Period.

Following inoculation by an infected mosquito, ten to twelve days are required for the onset of a paroxysm. In rare instances the incubation period may be as short as five to six days. This probably depends to some extent on the number of organisms inoculated. Malarial infection of the mosquito is not transmitted to the offspring the latter,* hence the bites of young mosquitos do not convey the disease unless they also have sucked malarial blood. The conditions are different in relation to Texas fever, in which the infection is transmitted by the female tick to her young.

Virulence.

The æstivo-autumnal parasite apparently is more virulent than the tertian or quartan. Not

* This is questioned by Schaudinn.

all cases of tertian or quartan fever are equally severe, and these variations may depend on differences both in virulence and in the resistance of individuals. When all the parasites divide within a period of two to four hours, the paroxysm is more intense but shorter than when division extends over six to eight hours (Ruge in relation to tertian fever). Some of the severer symptoms are due to the localization of the parasites (brain and intestines), rather than to special toxicity.

Relation of Symptoms to the Biology of the Parasites.

The melanemia of malarial fevers is due to the fact that the parasites absorb the hemoglobin from the erythrocytes, transform it into melanin by their metabolic activities and liberate the melanin at the time of cell division. The anemia results from destruction of the erythrocytes.

The cause of the fever and its periodic recurrence is more difficult to explain. As stated above, the fever begins in both tertian and quartan fevers at the time division forms of the parasites are encountered in the peripheral blood. Although all the parasites do not divide simultaneously, the process is complete within a period of four to eight hours and the paroxysm begins early in this period. It is quite natural, then, to infer that by the division of the parasite and the escape of the young cells from the erythrocytes, toxic substances are thrown into the circulation, and that the febrile reaction is due to the action of these toxins. Methylene blue has the power of preventing segmentation of the parasites (Ehrlich), and it has been shown that the paroxysm of fever may be averted by administering methylene blue at the proper time. This corroborates the view that the segmentation of the parasites causes fever in some

Fever and Schizogony.

way. The paroxysm would seem to represent the time required for the exhaustion of the toxins set free at the time of the cell division.*

Duration of Paroxysms.

On the basis of the conditions just cited, the brief duration, sharp limitation and regular recurrence of the paroxysms in tertian and quartan fevers become intelligible. In a similar manner the longer paroxysms and shorter intermissions which characterize the typical æstivo-autumnal infection (i. e., in first attacks) are related to the habits of division of the corresponding parasite. All the cells do not divide within a relatively short period, as in tertian and quartan fevers, but the process of division rather stretches out over from twenty-four to forty-eight hours. This accounts for the longer duration of the paroxysm. When the last cells of one generation are dividing, perhaps after the fever has gone down, the first cells of the succeeding generation are well on toward maturity and their division within a short time inaugurates a new paroxysm; the brief intermission would seem to be explained by this condition. As the disease lasts longer, or as relapses develop, the periods of division of the parasite are less sharply limited and a course with an irregularly continuous (?) fever may be established.

Quotidian Fever.

Quotidian malarial fever is caused either by a double infection with tertian parasites or a triple infection with quartan parasites. In either instance a generation of parasites matures and divides every twenty-four hours. The cause of

* Rosenau, Parker, Francis and Beyer produced a typical paroxysm in a healthy person by injecting filtered serum taken from a tertian patient during the chill. This was intoxication, not infection.

the double or triple infection is not known definitely. In some instances it is possible that successive inoculations by different mosquitoes has occurred. On the other hand a fever which is primarily tertian or quartan may gradually change into the quotidian variety, and in this condition it is possible that the organisms may gradually separate themselves into two or three distinct generations, which reach maturity on successive days.

Mixed Infections.

In other instances mixed infection with two kinds of parasites is encountered. This is usually æstivo-autumnal fever combined either with tertian or with quartan. Either the æstivo-autumnal may be primary on the one hand or the tertian or quartan on the other. The clinical course is complicated correspondingly. It is doubtful if tertian infection is ever mixed with quartan. Ruge speaks of experiments by Dr. Mattei which indicate that a mixed infection does not continue indefinitely as such. A patient suffering from quartan fever was inoculated with æstivo-autumnal blood; in time all the quartan parasites disappeared, leaving only the æstivo-autumnal.

In malarial cachexia there is not only an insufficiency of the blood-forming organs, but other parenchymatous organs have suffered as a result of prolonged intoxication. The blood-forming organs can not keep pace with the destruction of the erythrocytes.

Trigeminal and supraorbital neuralgias and periodic headaches occur sometimes as accompaniments of malarial infection, even when there is little or no fever, and no parasites may be discoverable in the blood. That they are malarial in origin is concluded from the fact that they subside under quinin treatment.

Cerebral and Intestinal Symptoms.

In some forms, and particularly in æstivo-autumnal fever, cerebral symptoms (e. g., coma) are marked by accumulations of the parasites in the small vessels of the brain; the vessels may be completely occluded. The conditions are similar in the small vessels of the intestines in malarial diarrheas.

"Black-water Fever."

The so-called "black-water fever," or hemoglobinuric fever, is not a special form of malaria, but a complication which, it is thought, is precipitated by insufficient or improper administration of quinin (Koch and others). It is most frequent in the tropics, hence in æstivo-autumnal fever, but may occur in the tertian and quartan types. Various observers have found that in all the way from 56 per cent. to 97 per cent. of the cases quinin precipitated attacks. Stephens and Christopher were not able to exclude quinin as a factor in any of the cases they encountered. The essential process is a massive destruction of the erythrocytes which is entirely out of proportion to the number of cells occupied by parasites; few or no parasites may be present. The amount of hemoglobin thus liberated is so great that it is excreted largely by the kidneys; anuria may result from occlusion of the tubules by pigment. How the quinin, or the quinin plus parasites, produce this extensive hemolysis is entirely obscure; the effect is that of an intense intoxication, in which the erythrocytes suffer primarily and chiefly.

Epidemiology.

The essential epidemiologic features of malaria are the following: It prevails especially in tropical and subtropical zones and less in temperate zones. It is most abundant in low, swampy regions, and in other places which afford quiet streams, ponds

or other standing water. Malaria is not directly contagious. In order to become infected it is necessary, customarily, for one to enter or be in close proximity to a "malarial district." That the virus is not carried far from an infected district is shown by the exemption of the crews of vessels which lie within two or three miles of such a district. Infection has long been supposed to take place chiefly by night. The disease may be introduced into new regions (of suitable climate) by the importation of malarial subjects. These and many other phenomena of malaria which were once very obscure have been cleared up by the mosquito theory.

Anopheles. There are many species of anopheles and they are distributed throughout the world in warm and moderate climates. *Anopheles maculipennis* is the most numerous species, and for it, as well as for *Anopheles punctipennis,* Howard has found several natural breeding places in this country. It is probable that many, but not all, species of anopheles may transmit malaria. The female only is a blood-sucker, the male living on vegetable material exclusively. After the female has obtained blood from man or another mammal it flies to a suitable pond or other collection of water, where it deposits its eggs.

Development "The adult mosquito lays its eggs on the surface of the water. The eggs float on the water for some days (two to four), after which they hatch and permit the escape of the larva.

"The larva is a free-swimming, worm-like animal, which eats greedily and grows rapidly, casting its skin several times in the process, till it reaches its full development. At this stage it sud-

denly changes its form; casting its skin, the worm-like larva assumes a comma shape and so becomes the pupa or nympha.

"During the pupal period the insect ceases to eat; profound anatomical changes take place within the pupal skin, whereby the masticatory mouth-parts of the larva are converted into the suctorial apparatus of the adult insect or imago. After a certain number of days the pupa case ruptures and the adult insect is liberated, furnished with wings and legs adapted for a life in the air." (James and Liston.)

In one instance Howard found the life cycle of *Anopheles maculipennis* to be: "Egg stage, three days; larval stage, sixteen days; pupal stage, five days, making a total period in the early stages of twenty-four days." The rapidity with which this process takes place depends largely on the temperature; it is more rapid in the hot weather of July and August than in the cold days of May. Anopheles usually does not lay its eggs in tin cans or barrels of water, but preferably in more open or cleaner water. Excavations which have become filled with water are favorable places, as are also collections of water from springs.

Migration of Anopheles.

The anopheles leads an adult life for many months and may even hibernate under suitable conditions either in the adult or larval form. It is generally stated that the insects do not fly more than half a mile from their breeding and feeding grounds. Their dispersal certainly extends beyond these limits, however. James and Liston enumerate the following methods of dispersal: 1. By direct flight over considerable distances. 2. By the eggs and larvæ being carried in streams and

canals. 3. By a multiplication of successive short flights by adults. 4. In conveyances.

Anopheles avoids high winds and rains, seeks shelter on excessively hot days and feeds and bites chiefly or only after sunset and before sunrise. The latter habit confirms the old belief that malarial infection occurs chiefly at night.

For further details as to the morphology and habits of the insect in its different stages, and for differentiation of the different genera and species, one should consult a textbook of entomology, or, for example, the book on "Mosquitos," by Howard (McClure, Phillips & Co., New York).

Prophylaxis. Individual prophylaxis may be accomplished and maintained by taking small daily doses of quinin, or larger doses (1 gram) every few days. One who has had malaria may likewise prevent recurrence by suitable quinin treatment. Quinin has the power of preventing division of the parasites, and, therefore, the power of preventing the paroxysms. "R. Koch's procedure consists in this, that one takes a gram of quinin every tenth and eleventh day, and if fever still develops, every ninth and tenth day." (Ruge.)

Other points in individual prophylaxis are, first, the application of ethereal oils (clove oil, oil of pennyroyal) to the exposed skin, and, second, the use of mosquito netting.

General Measures. The important practices for general prophylaxis are the following: 1. The draining of swampy places and of pools of water where anopheles may deposit its eggs. This in many instances manifestly can not be accomplished. 2. Covering pools of water with petroleum. This is to a degree successful. Every square meter requires ½ liter of pe-

troleum (Kerschbaumer), and the oil must be added fresh every seven or eight days. The layer of oil excludes the air from the larval mosquitoes and they drown. If fresh oil is not added occasionally new eggs may hatch. 3. Koch's method of extermination of malaria. This consists of the searching out of all cases of malaria and the destruction of the parasites by appropriate quinin treatment. Koch practiced this method in an infected locality of New Guinea and in a relatively short time freed it of malaria. If all the plasmodia in a community are destroyed the disease can not again become endemic unless it is introduced from without or unless infected mosquitoes are imported. Manifestly this method must be practiced on an extensive scale in order to render it permanently successful. It seems to have been demonstrated, however, that the number of cases in any given locality may be materially decreased by pursuing it.

Immunity.

So far as is known, susceptibility to malaria is universal. The belief is very general that one attack of malaria not only does not protect against reinfection, but even predisposes to it. Two facts, however, show that acquired immunity (relative or absolute) is possible. First, in certain regions of Africa where malaria is endemic the adult natives rarely suffer from the disease, and then only from light attacks, whereas European visitors contract the disease in severe form. The cause of this immunity was explained by Koch. "Koch found that the native adults of malarial countries were free from malaria, but that the children suffered almost universally from malarial diseases. If they recovered from the original infection they

became immunized in time through continued new attacks or relapses, the number of malarial children gradually decreased with their age, and in the vicinity of the tenth year the only evidence, in general, of a previous infection was an enlarged spleen, and even this disappeared during puberty, so that the adult natives finally appeared as healthy and malaria-immune persons." (Ruge.) The objection raised by many that such immunity is not observed in Italy and other civilized countries where malaria is endemic, is met by the fact that the disease in these countries is not permitted to run an uninterrupted course. Treatment with quinin is instituted and the immunizing process is thereby broken off. Koch also established that immunity against one type of parasite is not efficient against other types.

Second, in civilized countries it has often been noted that subsequent attacks are of a milder character than the primary; the disease may in time "wear itself out," even without quinin treatment. Ruge gives as an accompaniment of this immunizing process the occurrence of the sexual cells in large numbers, even up to 50 per cent. of the total number of parasites (tertian fever). In such cases large numbers of the parasites die before they reach maturity, their death being indicated by shrinking and clouding of the cells and alterations in or disappearance of the chromatin. It is somewhat characteristic of quartan fever, and still more so of æstivo-autumnal, that the sexual cells are much more numerous in recurrences than in primary attacks. One may be able to differentiate a relapse from the primary attack by the number of sexual cells encountered (Ruge).

Nothing in the way of serum therapy has been accomplished, and it is doubtful if any serum could equal quinin in efficacy.

MALARIA OF BIRDS.

Proteosome.

What is considered as true malaria also occurs in birds. One of these diseases is caused by a proteosome (*Proteosoma Labbé, Cystosporon danielewsky, Hemameba relicta*). Sparrows, hawks, buzzards, crows and pigeons are affected. Like the malarial parasites in man, the parasite enters the erythrocytes and has both a sexual and an asexual cycle of development, the latter taking place in the infected animal, the former in the stomach of the common mosquito (*Culex pipiens*). Hence in its development proteosoma is perfectly analogous to plasmodium. The disease is transmissible from bird to bird by the inoculation of infected blood.

Halteridium.

Halteridium is still another hemosporidium which infects birds. It was in the study of this organism that MacCallum first saw the phenomenon of impregnation. All the cells seen in the blood appear to be divisible into male and female, and although MacCallum had seen impregnation in microscopic preparations the life cycle for a long time was obscure. Recently Schaudinn has found that the sexual cycle is completed in *Culex pipiens*. He considers the organism to be a trypanosome. "I have been able to prove that the halteridium is the sexual stage of a trypanosome which multiplies in the common mosquito—*Culex pipiens*—and after a complicated migration through the body of the mosquito is again introduced by its bite into the blood of the owl, where, after a period of sexual multiplication, it is transformed into the well-known male and female halteridium."

II. TRYPANOSOMIASIS.

Genus Trypanosoma.

Gruby created the genus *Trypanosoma* in 1843, when he gave the name of *Trypanosoma sanguinis* to a flagellate protozoon which he found in the blood of frogs. Since that time similar organisms have been found in the bloods of many animals and the genus *Trypanosoma* has grown to considerable

dimensions. It is not improbable, however, that a number which now bear independent names will be shown to be identical. This suggests itself particularly in relation to trypanosomiasis in horses, in which the infections are known under four separate names in different countries, and the parasites are given separate specific names. The study of these infections is so young and has been prosecuted in such widely separated countries that the existing chaos is quite natural and can be adjusted only as time and circumstances permit of close comparative study. Until such a time the prevailing views as to independence of species and of infections must be recognized.

Morphology. Trypanosomas vary a great deal in size and morphology. Roughly, they are from one to five microns thick and fifteen to forty-five microns long, including the flagellum. All species possess active eel-like movements, some traveling rapidly, others slowly. A long, actively-motile flagellum projects from the anterior end, and where it joins the cell body is continuous with an "undulating membrane," which extends along a border of the organism to a point near the centrosome or micronucleus in the posterior portion of the cell. The centrosome is sometimes spoken of as analagous to the "eye spot" of some other protozoa. The undulating membrane is more or less wavy or folded and its breadth varies. The centrosome presumably has a close relationship to the undulating membrane, and, through the latter, with the flagellum. The nucleus is in the anterior portion of the parasite. In relation to some species a contractile vacuole is spoken of. An endoplasm and an ectoplasm may be differentiated.

Division. Division of trypanosomes is nearly always longitudinal, rarely transverse. According to Plimmer and Bradford, conjugation may occur, "followed by an ameboid stage and division by segmentation." The ameboid stage at times occurred independently of conjugation. (Musgrave and Clegg.)* In the process of longitudinal fission the order of division of the different parts of the cell is as follows: 1, Centrosome; 2, flagellum; 3, nucleus and protoplasm (Laveran and Mesnil). After division has occurred the two cells may remain attached at their posterior ends for some time. By a repeated division of young cells, the posterior ends remaining attached, rosettes are said to be formed. Others consider rosette formation as a phenomenon of agglutination. Possibly both phenomena occur.

Differentiation. Trypanosomas are differentiated on the basis of size, pathogenicity, motility and certain points in morphology, as the position and size of the micronucleus or centrosome, the presence of a contractile vacuole, the size and tinctorial qualities of the nucleus, the degree of granulation of the protoplasm and the location of the granules, appearance of the undulating membrane, length of flagellum and shape of the posterior non-flagellated end.

TRYPANOSOMIASIS IN MAN.

Trypanosomatic Fever. Nepreu in 1898 first found trypanosomes in the blood of man in Algiers in eight cases. His observations were passed over temporarily. The parasite bears his name (*T. neprevi*). Again in 1901 Forde discovered similar parasites in Western

* Knowledge is very deficient concerning the questions of conjugation and sexual cycles. An exception is to be made in relation to halteridium, a parasite of bird malaria, which is a trypanosome, according to Schaudinn. (See page 483.)

Africa (Gambia), and since that time a number of cases of "Gambian fever" or trypanosomatic fever in man have been imported. In this instance the parasite was called *T. gambiense* by Dutton and *T. hominis* by Manson. The disease is said to follow the bite of a tsetse-fly (Glossina palpalis), at least in some instances. The tissues around the bite become inflamed and in from a few days to two weeks recurring attacks of fever set in, and a patchy and ringed erythematous eruption appears on the skin. Forde gives as the chief clinical findings in his case: 1, the irregular intermittent temperature; 2, the edematous condition of the face and lower extremities; 3, the rapid and variable pulse and respiration, unaccompanied by any evident cause; 4, loss of weight, with marked debility, wasting and lassitude; 5, the persistence of these symptoms and their resistance to treatment. The parasites are most numerous in the blood at the time of the febrile attacks. Recovery has not been reported.

Sleeping Sickness.

Sleeping sickness has been endemic in certain districts of Africa for a long time, and, although confined to a very limited district at one time, it appears now to have extended to distant parts. Speaking of trypanosomatic fever and sleeping sickness collectively, Ruata says that while originally confined to a small district in Western Africa between the latitudes 15′ North and 15′ South, it is now found one thousand miles up the Congo (Bangola, Stanley Falls) and in East-central Africa on the shores of the Victoria Nyanza Lake. "Now it extends from the mouth of the Katonga River through Uganda (1901, Cook), Kome Island, Busaga, Buvuma, Kavirondo, Kisumu, Lumbwa,

Homa, Kasagunga, Lusinga Island, the eastern shores of the lake, joining the south of the boundary river Gori in the Udemi district of the Sultina of Obo" (Ruata).

Its extension supposedly has been facilitated by rapid transit. "The disease is most prevalent amongst the inhabitants of low-lying shambas (banana and potato plantations) in places along the shores of the Victoria Nyanza, or in wooded districts not far from the water" (Christy). Those living on high ground are much less infected than those living in the low moist places near water. A great deal of stress is laid on its close association with inland bodies of water. **Occurrence.**

It apparently has no relation to sex, age, seasons, food or drinking water, and is related to occupation only in so far as the occupation carries one into the low places mentioned.

Trypanosomes in Sleeping Sickness.

At one time (1891) Manson advanced the idea that sleeping sickness is caused by the minute *Filaria perstans*. It has since developed that this parasite occurs in 70 per cent. of the natives in certain districts, and that sleeping sickness may occur in areas in which *Filaria perstans* does not exist; Manson has abandoned this view. A number of investigators also found cocci in the cerebro-spinal fluid, but this occurred very rarely during life and at a late stage of the disease; such organisms are probably secondary or agonal invasions in spite of their rather frequent occurrence. Further investigations by Castellani disclosed the presence of a trypanosome (*T. castellani*) in the cerebro-spinal fluid of a large percentage of the cases, and a little later Bruce found this organism in all the cases he had examined. This observation has

been confirmed so many times that the trypanosome is now generally considered as the cause of the disease.

Tsetse Fly. Sleeping sickness is not contagious in the ordinary sense, and Bruce furnishes rather strong evidence that it is transmitted by the bite of a tsetse-fly (*Glossina palpalis*). The distribution of the disease corresponds to the habitat of this fly, and Bruce transferred the infection to monkeys by means of flies which had bitten those suffering from sleeping sickness. Sambon does not consider the experiments above criticism.

Symptoms. A pronounced lethargy or somnolence is the most striking clinical feature of the disease. "The appearance of the somnolent condition is preceded, often for a long time, by prodromal signs, which are so characteristic that the patient's neighbors cannot possibly be deceived as to the fate that awaits him. The victim complains of weakness, langour, dejection, disinclination for work, headaches, particularly localized over the occiput, a sensation of weight in the head and giddiness. His eyelids tend continually to close and he has a tendency to go to rest at unusual hours of the day; for this purpose he seeks out lonely quiet spots, where he spends a long time in dozing" (Scheube). For some time he is able to resist the somnolence, and when aroused gives intelligent answers. He eventually acquires an unsteady gait and walks about like a drunken man. The temperature of the body appears to be lowered, although irregular attacks of fever occur. The somnolence gradually becomes more intense, the patient grows very weak, the pulse small and thready, respiration difficult, the edema seen in tryansomatic fever is rather

constant, incontinence of the urine and feces may develop; the patient commonly dies after passing into a state of deep stupor. Convulsions and paralyses are noted; the mind usually is clear when the patient is conscious, although maniacal attacks and delusions are occasionally noted. The cervical and superficial lymphatics are frequently but not constantly enlarged. A papulo-vesicular eruption is quite characteristic and persistent and the skin becomes very dry. The incubation period varies from six to eighteen months, and the somnolent state from three to twelve months. Recovery rarely occurs.

Meningo-Encephalitis.

The essential anatomic change is meningo-encephalitis, the soft membranes being thickened, containing a milky fluid and the vessels of the pia and brain being surrounded by an extensive infiltration of mononuclear leucocytes.

Identity of Trypanosomatic Fever and Sleeping Sickness.

The discovery of trypanosomes in sleeping sickness suggested that tryanosomatic fever may really represent the long prodromal stage of sleeping sickness. This view has been greatly strengthened by a case reported by Manson in which a typical case of tryanosomatic fever was seen to pass into typical and fatal sleeping sickness. The wife of a missionary in upper Congo was bitten by a tsetse-fly, and following an inflammatory reaction at the seat of the bite, she developed and ran a long course of tryanosomatic fever. After a year and a half to two years of remittent attacks of fever, the organisms being found in the blood repeatedly, she grew weaker, became somnolent and died in a comatose condition. The anatomic changes at autopsy were typical of sleeping sickness. Some who are not quite willing to accept the

unity of the two diseases suggest that the sleeping sickness may have been superimposed on trypanomatic fever.

Assuming that the two conditions represent different stages of the same disease, we would have to recognize trypanosomatic fever as the first stage and the lethargy of sleeping sickness as the second. If this proves to be correct the name of *T. neprevi* should be retained for the organism and the other names dropped (*T. gambiense, T. hominis, T. castellani*).

The Parasite.

It is believed that *T. castellani* is a distinct species of trypanosome. It is hardly possible to associate it with nagana, since sleeping sickness and nagana do not coincide in their distribution, and, moreover, the morphology and pathogenicity of *T. castellani* differ from that of *T. brucei.* The former is not infectious for the "donkey, ox, guinea-pig, dog, pup, goat and sheep" (Ruata). *T. castellani* is from 18 to 25 microns long and 2 to 2.5 broad. Its morphology in general is like that of other trypanosomes, although there are sufficient differences to establish its independence. Its motility is rather slow, and in contrast to other trypanosomes it moves in the direction of its non-flagellated end. The failure to find any distinctive difference between this organism and *T. neprevi* (*T. gambiense*) is an additional point in favor of the unity of trypanosomiasis fever and sleeping sickness.

TRYPANOSOMIASIS IN ANIMALS.

On account of the prevailing general interest in the subject, the more important trypanosomatic infections in animals and the corresponding parasites will be sketched briefly.

General Symptomatology.

Musgrave and Clegg speak of certain general symptoms which are common to surra, nagana, *mal de cederas* and dourine, as follows: "After an incubation period, which varies in the same class of animals and in those of different species as well as with the conditions of infection, and during which the animal remains perfectly well, the first symptom to be noticed is a rise of temperature, and for some days a remittent or intermittent fever may be the only evidence of illness. Later on the animal becomes somewhat stupid; watery catarrhal discharges from the nose and eyes appear; the hair becomes somewhat roughened and falls out in places. Finally the catarrhal discharges become more profuse and the secretion more tenacious and even purulent; edema of the genitals and dependent parts appears; a staggering gait, particularly of the hind parts, comes on and is followed by death."

Infectiousness of Blood.

The incubation period varies from a few to several days. Pronounced anemia develops, the method of destruction of the erythrocytes being unknown. Lymphatic enlargement is the rule, and during the incubation period the parasites probably undergo great proliferation in the lymph glands. It is somewhat characteristic that massive invasion of the blood stream occurs periodically. With a paryoxism of fever their numbers increase in the blood, and during the intermission they decrease and may be so few as not to be found microscopically. Even when few or no parasites are found in the circulation, however, the blood usually is infectious for other animals. During the intermissions it is possible that they are largely within the lymph glands or other internal organs. The cause of these variations is not known, and it can not be said now that they are related to cycles of development like those of the malarial parasites. Voges suggests that they may represent the establishment of successive periods of temporary immunity (*mal de cederas*). These are only general features, and variations occur in infections in different animals and by different parasites.

Trypanosomiasis of Rats.

Trypanosoma lewisi, recognized in the blood of the rat by Lewis in 1879, and given its present name by Kent in 1882, infects wild rats throughout the world, and in some localities a very high percentage of the animals are

infected. The parasite is readily found in the peripheral blood (as from the tail), where a large number may be present in a single field of the microscope; sometimes, however, prolonged search is necessary for their discovery. Its dimensions vary: 1.4 to 3 microns in diameter, 10 to 25 microns in length, according to different observers. It is of lancet-form, possesses a finley granular endoplasm and a clear ectoplasm, and from the latter spring the flagellum and the undulating membrane. "The former (flagellum) is about as long as the body itself; it originates at the posterior end of the animal in a granule-like structure, called the flagellar root, extends forward as a marginal thickening of the undulating membrane and becomes free only at the anterior end of the animal from which it extends into the surrounding medium as a flagellum" (Doflein). At its posterior extremity the parasite ends in a sharp point. In its anterior portion it contains a strongly staining nucleus; a contractile vacuole is not described. Its motility is, perhaps, more active than that of any other trypanosome, and in a fresh mount of rat's blood it may move across the field so rapidly as to be followed with difficulty.

Division takes place by longitudinal fission (rarely transverse), and by repeated division rosettes are formed.

Cultivation. Novy and McNeal succeeded in cultivating this organism artificially on a medium consisting of rabbit's blood, 2 parts, agar, 1 part. The growth occurs in the condensation fluid, and the organisms were carried through many generations. In cultures they vary greatly in size (from 1 to 60 microns in length). "The existence of the small forms accounts for the fact that we have repeatedly been able to infect rats with Berkefeld filtrates of such cultures." It is remarkable that so many of the rats which harbor the parasites appear to be perfectly healthy. However, the animals not infrequently die from the infection, and in some instances fairly severe epidemics have been noted. The infection is found also in the hamster, a European rodent, and in white rats. White mice are susceptible to inoculation (Doflein).

Trypanosoma brucei, found by Bruce in 1894 in the blood of animals suffering from nagana or the tsetse-fly disease, in Zululand, is somewhat different morphologi-

cally from *T. lewisi,* being more worm-like in form, having a blunt posterior extremity, less motility and greater pathogenicity. "The undulating membrane is broader and more plicate, the protoplasm colors more easily and more deeply" than in *T. lewisi.* Its length is said to vary, depending on the animal which harbors it, being largest in the rat and shorter and thicker in the dog. Its dimensions as given by Laveran and Mesnil are 1 to 1½ by 26 to 27 microns. Its structure is similar to that of *T. lewisi,* containing a nucleus near the middle of the body and a deeply staining centrosome in the posterior portion in or near which the flagellum has its origin. A contractile vacuole lies anterior to the centrosome. **Nagana.**

Natural infection (nagana) with this organism occurs in horses, cattle, mules, and also in some wild animals, as camels, buffaloes and hyenas. It is, however, a tropical disease, occurring chiefly in various parts of South Africa. Nearly all animals are susceptible to artificial infection by the injection of diseased blood.

The distribution of nagana corresponds with the distribution of the tsetse-fly, and Bruce discovered that this fly, after feeding on the blood of an infected animal, transfers the disease to others by biting. Horses, asses, cattle and hogs were infected artificially in this way, but man appears not to be susceptible. It is assumed, but perhaps not definitely proved, that no other fly or insect transmits the disease. Immediately after it has fed on infected blood it is capable of transferring the disease; hence, further development of the parasite in the tsetse-fly is not essential for its continued infectiousness, and, indeed, it is not certain that any further development occurs. **Tsetse Fly.**

Nagana presents a remittent or intermittent type of fever, catarrhal secretion from the nose and eyes, subcutaneous edema, particularly of the abdominal region, prepuce and posterior extremities, roughening and shedding of the hair, marked emaciation, weakness and anemia develop, and the animal dies in a state of exhaustion. The spleen is greatly swollen, the red corpuscles are diminished in number, and the urine may be blood stained. The parasites are found in enormous numbers in the blood.

The disease is almost invariably fatal. It may last

for weeks or months in horses, and even much longer in cattle. It occurs not infrequently in epidemic form, wiping out the horses and cattle of infected regions. In wild animals it is suggested that the disease may be more chronic, and the shifting of such animals may serve to introduce the infection to new regions, but only to such regions as harbor the tsetse-fly.

Cultivation. Novy and McNeal cultivate *T. brucei* on a medium similar to that used for *T. lewisi.* The former is more exacting in its conditions for growth, preferring a medium containing blood and agar in a ratio of two to one or three to one. Cultures were kept alive for at least one hundred days through eight generations, although virulence was soon lost.

Surra. *Trypanosoma evansi* is the name given by Steele to a parasite discovered by Evans (1880), in India, in the blood of horses suffering from surra. It has the same general morphologic features as *T. brucei,* with dimensions of 1 to 3.5 or 4 microns by 20 to 35 microns, including the flagellum (Musgrave and Clegg). It contains a nucleus and possibly a contractile vacuole. The whole posterior extremity is contractile, according to Musgrave and Clegg, and this may also be true of other trypanosomes. Its motility is moderate and eel-like. It differs from the trypanosome of rats (*T. lewisi*) in its larger diameter and in its greater pathogenicity; *T. evansi* is pathogenic for "nearly all animals." It is longer than *T. brucei.*

Surra affects horses chiefly, and has caused immense losses in India and in the Philippine Islands. In India it is certainly transmitted by certain flies, and the same probably is true in the Philippines. Musgrave and Clegg demonstrated also that fleas may be of great importance as carriers. By this means they were able to transfer the disease from dog to dog, rat to rat, and rat to dog. They frequently found the parasites in native rats and believe that this animal may serve as a host in which the disease is maintained. Cattle are susceptible to infection, but the disease is less malignant in them and runs a long course; hence, they may be an important factor in maintaining an epidemic. The disease is also transmitted from horse to horse. In India, camels, elephants

and buffaloes also suffer from the disease. Surra resembles nagana in its clinical and anatomic aspects.

Dourine.

Doflein gave the name of *Trypanosoma equiperdum* to an organism described by Rouget in horses and asses suffering from dourine. Laveran and Mesnil call it *T. rougetii.* According to Rouget, the parasite resembles *T. brucei* closely. Doflein (1901) states that a nucleus and vacuole have not been seen. Dourine occurs in Algiers, southern France, Navarre and in the Pyrenees districts of France and Spain. The infection is transmitted by coitus and is limited largely to animals which are used for breeding. Ulcerations, particularly of the genitals, are characteristic. That it is not transmitted by insects may be due to the absence of suitable insects from these localities. The identity of dourine with surra or nagana is not yet determined. It is said to be more chronic than surra. Doflein recognizes the organism as an independent parasite. Infection may be transferred to dogs, white mice and other animals.

Mal de Cederas.

Trypanosoma equinum (Voges) or *T. elmassianii* is the parasite found in *mal de cederas*, a disease of horses in South America, resembling surra, nagana and dourine.

Infections of Other Animals.

Two different species have been found in the blood of South African cattle: *T. theileri* (Bruce, 1902) and *T. transvaaliense* (Laveran and Mesnil, 1902). The characteristic feature of the latter is the location of the centrosome near the nucleus near the center of the parasite. The following trypanosomes are found in fish: *T. cobitis, T. carassii, T. remakii, T. soleæ, T. borrellii;* the following in birds: *T. avium, T. eberthii. T. balbianii* occurs in oysters, *T. rotatorium* in frogs.

Immunity

Between various animals and the different trypanosomes a number of examples of natural immunity are known. The extent to which man is susceptible to sleeping sickness is not known, but since the disease may occur in Europeans as well as in native Africans, it is probable that susceptibility is general. Laveran and Mesnil state that sheep, deer and cattle which have recovered from nagana have an active immunity to the disease,

and it is thought that the immunity of some animals (e. g., cow) may be increased by injecting infected blood. Koch, and also Schilling, have attempted to render trypanosomas suitable for vaccination by passing them through asses, and a certain degree of success was reported. The serums of actively immunized animals do not exert a pronounced protective or curative action, although they may in some instances prolong the incubation period. Human serum has a certain protective and curative power for rats and mice which have been inoculated with the parasite of nagana. In some instances immune and normal serums kill trypanosomes, as shown by rapid loss of mobility.

"Trypanroth."

A most interesting bit of experimental therapy is that of Ehrlich and Sachs in curing and protecting mice against *mal de cederas* by injecting and feeding "trypanroth," a synthetic dye. The dye was less efficient in experimental nagana and in trypanosomatic infections of rats, guinea-pigs and dogs. Among hundreds of other dye-stuffs no other was effective. The immunity and cure established in this way is very temporary and is to be referred to a reaction caused in the body rather than to a direct effect on the parasites.. The latter are not killed by the dye in test-tube experiments. "One may conceive of the action of trypanroth in this way, that as a result of a fresh injection of the dye a reaction takes place in the animal's body, which leads to the death of the trypanosomes; the reaction products possess only a temporary character and cease to be formed as soon as the dye is disposed of."

Laveran reports a favorable influence on trypanosomiasis in mice and rats by a combined treatment with sodium arsenite and "trypanroth."

Spontaneous agglutination and agglutination by normal and immune serums have been described. At the present time agglutination is of no value from the standpoint of diagnosis.

III. "SPOTTED FEVER" OF THE ROCKY MOUNTAIN STATES.

Limited Occurrence.

In the valley of the Bitter Root River of Montana, and in certain sections of Idaho, Wyoming and Washington an acute febrile disease, known in those localities as spotted fever, is encountered in the spring months. The disease is defined by Maxey as "an acute, endemic, non-contagious, but probably infectious, febrile disease, characterized clinically by a continuous moderately high fever, severe arthritic and muscular pains, and a profuse petechial or purpural eruption in the skin, appearing first on the ankles, wrists and forehead, but rapidly spreading to all parts of the body." (Cited by Stiles.)

Pyroplasmæ Hominis.

In 1902-03, Wilson and Chowning studied many cases of the disease in Montana, and described as the cause a protozoön organism which they consider as a pyroplasma (*Pyroplasma hominis*). The organism is a hematozoön, occurring within the erythrocytes. Young cells resemble the "hyaline bodies" of malaria, are of ovoid shape, 1 micron thick and 1 to 2 microns long, and usually occur in pairs, but sometimes in numbers of 4 to 16, within an erythrocyte. The smaller ends of pairs often are directed toward each other, and they may be connected by a fine filament. They occur both in the red corpuscles and in the plasma. As they grow larger, two to three by three to five microns, only one parasite usually is found within an erythrocyte, and in this stage they show active ame-

boid movement with the formation of pseudopodia. Eventually they assume a spherical form in fresh preparations. They were able to transfer the infection to rabbits by the inoculation of infected blood.

Transmission by Ticks.

After identifying the organism as a pyroplasma and having in mind the part that ticks play in the transmission of Texas fever, and perhaps pyroplasmosis in other animals (horse, sheep, dog), Wilson and Chowning directed their attention to the question of tick bites in those who become infected. It developed that of the 23 cases examined in 1903 all had been bitten by ticks, and fourteen had been bitten in from two to eight days before the onset of the disease. They concluded that the disease is transmitted in this manner.

Intermediate Host.

They also searched for some other host than man, in which the parasites might flourish continuously and constitute a source of infection for the ticks. This they believe was found in a certain gopher (*Spermophilus columbianus*). On the west side of the river—that side in which the disease attacks man—they found the erythrocytes of about 20 per cent. of the gophers infected with a parasite similar to that found in man. On the other hand, the blood of sixty-two gophers from the uninfected side of the river showed no parasites. "Early in the spring the spermophile is said to harbor great numbers of ticks." Similar parasites were found in no other species of animals.

Stiles, in later investigations, could not confirm the results of Wilson and Chowning, being unable either to find the parasites which they described in man, or to accept the tick-gopher hypothesis. Further investigation of this disease is needed.

TEXAS FEVER.

Texas fever of cattle may be considered briefly as a well-established example of pyroplasmosis.

The Parasite.

Th. Smith and Kilbourne (1893) discovered a pear-shaped protozoon (*Pyroplasma bovis*), which occurs in pairs in the erythrocytes of infected cattle. The parasite measures from 2 to 4 microns long by 1.5 to 2 microns broad, the smaller ends of the pairs lying in apposition. The organisms have a rapid but rather coarse ameboid movement. About 1 per cent of the corpuscles are invaded ordinarily, but in fatal cases the proportion may rise from 5 to 10 per cent. The method of proliferation of the parasite has not been followed out definitely. According to Smith and Kilbourne, numerous minute motile forms (coccus-like bodies) penetrate the corpuscles and eventually reach the pear-shaped form. The breaking up of the adult pear-shaped parasites into such small forms has not been observed.

A characteristic symptom of Texas fever is the pronounced hemoglobinuria which has given to the disease the additional name of hemoglobinuric fever.

The disease is transmitted by means of a tick (*Boophilus bovis*). The six-legged larvæ fill themselves with blood, and in about eight days have been changed into eight-legged nymphæ. In eight days more they have changed into fully-formed sexual animals, and, after filling themselves with blood and after having been impregnated, they drop off the cattle and lay their eggs. The eggs in from 3 to 4 weeks have again grown into larvæ, which are then ready to attach themselves to cattle (cited from Kossel). Inasmuch as infected ticks transmit the parasites to their offspring, the bites of the larvæ are able to give rise to the disease in cattle. A mature tick may deposit from 2,000 to 4,000 eggs. It has not been possible to transmit the disease to other species.

Transmission

The disease is endemic in the southwestern states, and the cattle in that region are supposed to acquire an immunity similar to that described by Koch in relation to malaria. Presumably the cattle first acquire the disease when they are young, and those which withstand it show resistance to the infection in later life. Cattle

from uninfected districts are more susceptible than those coming from localities in which the disease is endemic, and the latter even when apparently healthy may introduce the disease into new herds. This is done through transportation of the ticks.

Partially successful attempts at active immunization have been made, and in Australia this is practiced on a fairly extensive scale. Five to ten cubic centimers of blood, taken from an infected animal, during the course of the disease or after recovery has been established, are injected into non-immune cattle. The disease is thereby reproduced in the latter with typical parasites in the blood. If the blood is taken from animals which have recovered, a milder infection results than when the blood of an actively infected animal is used (Pound, cited by Kossel). The resulting immunity is not an absolute one, however, and the percentage of mortality is fairly high. According to Dodson, the serum of animals which have completely recovered has no protective power for other animals.

For prophylaxis it is important to free the cattle from ticks (as by an oil bath) and to avoid infected fields. If cattle are kept from an infected pasture for two years, the ticks die out very largely (Morgan).

IV. AMEBIC DYSENTERY.

Amebæ. Amebæ are unicellular animal organisms which contain one or more nuclei, a "contractile" vacuole, a granular endoplasm and a tougher more hyaline ectoplasm, having the power of locomotion by means of pseudopodia or by a gradual flowing forward of the cytoplasm. They nourish themselves by digesting bacteria and other lower organisms or solid particles of decaying matter, which they ingest after the manner of phagocytes. They proliferate by division of an adult cell into two daughter cells, and certain of them reach a cystic stage in which hundreds of endospores are formed (*Ameba proteus*). Some of them utilize higher animals as hosts only occasionally, while others

are known only as parasites. They frequently are encountered in the intestines of mice, frogs and other animals.

Distribution.

Amebæ are widely distributed in nature, existing to the depth of 2 meters in tropical soils, in the water of springs and wells and practically all surface waters (hot countries), and in stagnant or sluggish waters in higher altitudes. They exist on hay, fruits and vegetables of all kinds, especially those grown on or near the earth; e. g., beets and lettuce.

Resistance.

Encystation takes place under certain unfavorable conditions, and in this condition the parasites withstand a temperature of —15° C. for twenty-five days (Musgrave and Clegg), and desiccation for ten to fifteen months. A temperature of 50° C. kills the vegetative and encysted forms. Sunlight for three hours and the *x*-ray kill them readily in the vegetative form, but not so readily when they are encysted. Most chemical bactericides destroy them, although they show a particular resistance to alkalies, even 20 per cent. NaOH (Frösch), and strong acids. They resist the action of 0.2 per cent. HCl, i. e., the acidity of the stomach contents. Quinin (1/2500 of the hydrochlorate) is strongly germicidal for *Ameba coli.*

Cultivation.

Under artificial conditions amebæ proliferate in the presence of other micro-organisms, and suitable mixtures they may be kept alive indefinitely on slightly alkaline bouillon agar. The only condition in which amebæ are found unassociated with bacteria is in the liver abscesses which occur as a complication of amebic dysentery. It is true that the bacteria may have been present originally, but in their absence it is

supposed that enzymes normally present in the liver stimulate the growth and proliferation of the parasites. Amebæ show a peculiar selective property for certain bacteria, although their affinities may be gradually modified. *Ameba coli* apparently prefers those organisms which flourish in the human intestines (*B. coli, B. typhosus, Sp. choleræ, Staph. pyog. aureus*). Almost any strain will, however, grow with a variety of bacteria. Growth occurs only on the surface of the agar plates. When a pure strain of ameba is grown with a single species of bacterium the culture is spoken of as a "pure mixed culture."

Amebic dysentery is primarily a disease of the tropics, where the natural conditions are favorable for the growth of the amebæ and their conveyance to man.

Ameba Coli.

First found by Lambl (1860), then by Cunningham and Lewis (1870), the organisms were described more accurately and given the name of *Ameba coli* by Lösch (1875). Lösch recognized them as the cause of a chronic form of dysentery, but it was Kartulis, in particular, who found the amebæ constantly in the discharges and ulcers of the disease, and also in the liver abscesses which accompany the infection. Since amebæ demand the presence of living bacteria for their growth,

Pathogenicity.

their independent pathogenic nature has been questioned by many who assume that the bacteria are the primary agents in causing the intestinal lesions and that the amebæ are only incidental or secondary factors. Many others, and particularly Musgrave and Clegg, consider that amebæ have essential pathogenic properties and are the primary agents in producing amebic dysentery. By the feeding of encysted cultures grown with other or-

ganisms, Musgrave and Clegg reproduced the disease typically in many monkeys. In one instance the amebæ were fed in conjunction with cholera vibrios; typical dysentery developed and during the course of the disease the vibrios disappeared from the stools. The vibrio alone proved to be non-pathogenic when fed to monkeys, and on this account they held the amebæ to be the sole cause of the dysentery.

Lesions

The principal lesions occur in the large intestine, in which are found round or oval ulcers with infiltrated or undermined edges. The ulcers may increase in size, or coalesce with others, and cause the sloughing of large areas of the mucosa or even of the muscular coats. The organisms are found in the intestinal contents, on the surface of the ulcers, in the infiltrated base and edges, and in the underlying tissues. They have been found associated with both chronic and acute appendicitis. Amebic liver abscesses are not infrequent in those regions in which the disease is endemic. The organisms probably extend to the liver from the intestines through the lymphatic or portal vessels. Not infrequently the association of the amebæ with bacteria is missed in the abscesses, and in these instances a "cold" abscess containing much necrotic material and detritus is produced. If contaminated with bacteria the abscesses have a more purulent character.

Prophylaxis

Suitable prophylaxis against amebic infection is suggested by the known distribution of these organisms. Of principal importance is the use of filtered or boiled waters and the avoidance of uncooked vegetables in regions in which the disease is endemic, as in the Philippine Islands.

Immunity. From the fact that foreigners going into tropical countries are more susceptible to infection than the natives, it is concluded that the latter have some natural (or acquired) immunity to the disease. Children are said to be less susceptible than adults and in them the disease yields to treatment more easily. There is no serum therapy for the infections. The salts of quinin in strengths of 1-1500 to 1-750 are amebicidal when injected into the colon.

V. SARCOSPORIDIA.

Morphology. Sarcosporidia are unicellular parasites which are found within the muscle cells of some animals, but very rarely in man. They are more or less tubular or oval in shape and are frequently referred to as Miescher's tubules. Their size varies greatly and certain species may reach a length of two centimeters. When well developed they possess two capsules—a dense outer capsule, which is perforated with minute canals (?) directed toward the center of the parasite, and an inner thin hyalin membrane. Both represent differentiated ectoplasm (Doflein). The endoplasm, even in young cells, gives rise to numerous small nucleated spheres (pansporoblasts), which increase in size and each of which eventually becomes multinucleated and forms numerous kidney or sickle-shaped, nucleated sporoblasts. Each sporoblast finally gives rise or is changed into a well-characterized spore with a membrane and a nucleus. This process takes place first in the central part of the parasite, but eventually extends to the ends as well. The central part of the old parasites contains only the empty network of endoplasm, the

spores having disappeared, and a section at this point strongly resembles that of a tubule.

The parasites are nourished through osmosis. None of the forms have definite motility. When the parasite outgrows the muscle cell which contains it, it is freed and becomes an intercellular parasite. Rather vague references are made to tumor-like formation as a consequence.

Occurrence.

Sarcosporidia have been found only in vertebrates, particularly in mammals; most often in sheep and hogs, but also in the horse, ox, mouse, rat. The muscles adjacent to the alimentary tract are involved principally (esophagus, intestines, diaphragm and abdominal muscles) and on this account it is supposed that infection takes place through the intestines. The exact method of inoculation is not known.

Sarcocystis lindemanni (*Sarcocystis hominis* or *Gregarina lindemanni*) is the only sarcosporidium definitely identified in man. The parasites were as large as 1.6 millimeters long and 170 microns broad. They possessed a thin capsule, thickened at the ends. The spores were banana-shaped and eight to nine microns long. The organisms were found in the muscles of the larynx.

VI. BALANTIDIUM COLI.

B. Coli is an infusorian (ciliate), with a more or less oval body, mouth opening and a short pharynx, is covered rather uniformly with short cilia, and presents longitudinal striations. It contains a bean-shaped chief nucleus and a secondary nucleus and two vacuoles on the right side. It measures 70-100 microns in length and 50-70 in breadth. Proliferation is through simple division.

Conjugation has been noted. Involution cysts are spherical and surrounded by a dense membrane.

Pathogenic Significance. The parasite is found in the intestines of the hog as well as in man, and the former may be its normal host. It occurs also in sewage waters and has been found in drinking water. Infections have been noted in those having nothing to do with hogs. The organisms may reach the intestines of man in an encapsulated state (?). It is found in diarrheal conditions in man rarely, and the question is still open as to whether the parasite is able to cause enteritis independently or whether it merely aggravates and prolongs an enteritis due to other causes.

The cecum and colon show the principal changes at autopsy, and are of an inflammatory and ulcerative nature.

A smaller species, *B. minutum*, has also been observed in the intestines of man.

VII. CERCOMONAS INTESTINALIS.

Morphology. This organism is small and colorless, the form spherical or oval. The single flagellum is for the most part very large and is situated at the anterior end (in the direction in which the parasite moves); the posterior end is long drawn out and is subject to changes in form. Sharp pseudopodia are sometimes formed. The nucleus lies in the anterior half of the body, and either here or on the sides are one or more vacuoles. A mouth opening is not differentiated, but at the base of the flagellum food is taken in at a particular point through a vacuole. Proliferation takes place through conjugation, binary division and the formation of swarm spores (?) within encysted forms. They abound in fresh water and in infusions of grasses.

Significance.

They are not of great parasitic importance, although cercomonas has been found in the intestines, especially in inflammatory conditions (cholera, typhoid), in pulmonary gangrene, putrid pluritis, and several forms have been observed in other animals.

It is not yet certain that cercomonas may be an independent cause of enteritis.

VIII. TRICHOMONAS.

Morphology.

Rather small, of a general pear-shape, rounded or pointed anterior end, and possessing three or four long flagella. When only three flagella are present an undulating membrane surrounds the body like a spiral beginning at the base of the flagella and may prolong itself into a flagellum. The posterior extremity is moderately pointed, a nucleus lies in the anterior end, and toward the posterior are several non-contractile vacuoles. Methods of proliferation unknown (Doflein).

Species.

Two species are found in man. *Trichomonas vaginalis:* possesses three flagella and an undulating membrane, and is of large size (15-25 microns in length). It is found in the vaginal mucus, when of acid reaction, in a large percentage of women (Dolflein), particularly in vaginal catarrhs. It disappears in an alkaline reaction.

Trichomonas hominis s. intestinalis: also possesses three flagella and an undulating membrane, but is smaller than *T. vaginalis.* It is found as a parasite in the human intestines, particularly in diarrheas (typhoid, cholera, mucous colitis, etc.,) and inhabits especially the upper and middle portions of the intestines. It is evacuated in consideral numbers following administration of cathartics. It appears not to be of much pathogenic sig-

nificance, but finds in the liquid stools and in an alkaline reaction conditions which favor its proliferation. It may be transmitted as a contagion (Epstein).

Other Flagellates.

Other species of trichomonas occur in the intestines of different animals.

Other less important flagellates are: *Lamblia intestinalis,* found in the intestines of many animals and in man in Germany, Italy, Russia and Sweden; *Bodo urinarius* (*Cystomonas urinarius, Plagiomonas urinaria*), found in the urine in cystitis (Künstler).

IX. COCCIDIOSIS.

Life Cycles.

Coccidia are essentially cell parasites, preferring the epithelial cells of the intestines and liver, although they may be carried to other organs. They have an alternating asexual and sexual cycle of development. The young sickle-shaped and nucleated sporozoite penetrates an epithelial cell, grows in size, and the nucleus subdivides many times to form new young cells, which eventually escape again as sickle-shaped sporozoites. This asexual process is called schizogony. Several stages of schizogony may follow successively, but eventually the organisms lose their proliferative power unless they are fortified by a sexual cycle. In the sexual cycle (sporogony) some of the sporozoites become differentiated into larger granular cells (female) and others into smaller cells (male). Of these two cells the male eventually divides into many flagellated microgametes, each of which is able to penetrate and fertilize a female cell (macrogamete). The female cell then forms a capsule, becomes an oöcyst, divides into sporoblasts, each of which eventually forms sickle-shaped spores,

which when liberated are again called sporozoites. Several species are recognized, depending on the number of spores formed by the oöcyst. In some instances the spore formation takes place in the outer world, and when the oöcysts are ingested the sporozoites are liberated. **Species.**

Coccidium cuniculi s. oviforme is a frequent parasite in the intestines and liver of the rabbit, occurs occasionally in the same organs in man from association with rabbits (?), and causes a hemorrhagic dysentery in the cattle of some countries (Switzerland). Horses, goats and swine may also be infected.

Spore formation takes place outside the host. The oöcyst is discharged in the feces and produces four spores, each of which forms two sporozoites. A new host is infected by the ingestion of spores.

Diarrhea and emaciation result from infection of the intestines, and in the liver cheesy nodules (coccidia nodules) are formed, containing parasites, degenerated cells and proliferated epithelium. A papillomatous proliferation of the epithelium of the bile passages and intestines may be produced. **Results of Infection.**

Coccidium bigeminum, a coccidium in which the oöcyst divides into two spore-containing cysts, has been found in man several times.

GROUP VI. DISEASES OF DOUBTFUL OR UNKNOWN ETIOLOGY.

I. HYDROPHOBIA.

Following the investigations of Pasteur, in which it was found that the virus of hydrophobia exists in the central nervous system in pure culture, the conditions seemed favorable for the discovery of the specific agent. As in the case of many other diseases, various bacilli, cocci, yeasts and so-called protozoa have been described as the cause, but satisfactory proof of their etiologic rôle has not been provided.

Bodies of Negri.

Among the recently described parasites (?) certain protozoon-like bodies (Negri bodies) found by Negri in the ganglionic cells are of a suggestive nature. Their average diameter is about five microns, but it varies between one and twenty-seven microns. They possess a "round, oval, elliptical, or coarse triangular form" (Marx), are differentiated into a central granular and a peripheral structure and may be surrounded by a doubly-contoured membrane. Negri considers these bodies specific for hydrophobia and reliable as a basis for anatomic diagnosis. They are found particularly in the pyramidal cells in the cornu Ammonis, the cells of Purkinje in the cerebellum, and the large cells of the cerebral convolutions. Many others have confirmed the findings of Negri. Against the hypothesis that these bodies are the cause of hydrophobia, the following points are cited: The distribution of the Negri bodies does not correspond with the greatest concentration of the virus

in the nervous tissue, the latter being most abundant in the medulla and pons where the Negri bodies are encountered rarely. They are not found invariably in animals dying of hydrophobia. They present certain analogies with "protoplasmic inclusions" seen in other conditions, as in carcinoma, variola, etc. Remlinger found that the virus passes through appropriate Berkefeld filters, and for this reason Schüder holds that the bodies of Negri, being too large for filtration, can not be considered as the specific organism. The view of Schüder may be criticized, since the smallest Negri bodies are so minute that their filtration would seem to be possible. Nevertheless, it must remain doubtful whether bodies one micron in diameter, the proliferation of which has not been proved, may be considered as parasites. The hypothesis of Negri is hardly on a satisfactory basis at present. Remlinger considers the bodies as "involution forms" of the tissue cells which have been invaded by the true parasite.

Filterability of Virus.

The filterability of the virus argues for its microscopic size. By means of filtration one may isolate it even from brains which are badly decomposed, and the method renders it possible to obtain pure cultures for purposes of immunization. Inoculation with filtered virus is sometimes followed by a prolonged incubation period which may depend on the retention of many of the organisms by the filter. A similar effect was produced by Högyes by inoculating with diluted virus.

By prolonged centrifugation of an emulsion of infected nervous tissue the overlying fluid loses its infectiousness.

The possibility that the organism secretes a sol-

Toxin.

uble toxin is important from the standpoint of immunization. A number of observers, particularly Babès, and Heller and Bertarelli, noted that filtrates of infected nervous tissue sometimes cause emaciation, paralyses and eventual death without producing a disease which is transmissible to other animals. The organism is without doubt toxic, but these results give us no idea of the nature of the toxin.

Resistance of Virus.

The virus of hydrophobia as contained in the central nervous system of infected animals exhibits strong resistance to chemical germicides. Five per cent. carbolic acid destroys it in fifty minutes, 1 per cent. in three hours, and 1-1000 corrosive sublimate in three hours (Marx). It resists the action of putrefactive bacteria, and has been found virulent in animals which had been buried for two to four weeks, even when the brain was putrid. Direct sunlight destroys it, however, in a very short time. According to Tizzoni and Bongiovanni, the rays of radium have a destructive action on the virus. It is less resistant to heat, being destroyed in one-half hour at a temperature of 52-58° C. (Högyes), but is not affected by the temperature of liquid air for three months. Chlorin destroys it very rapidly. It is gradually weakened by desiccation, as first shown by Pasteur, the virus probably undergoing gradual death rather than mere attenuation. It is said to be attenuated by the action of the gastric juice and by the bile. When the nervous tissue is emulsified in glycerin, virulence is retained for months (Roux). On the other hand, glycerin appears to destroy the virulence of filtrates (Di Vestea).

Pasteur gave the name of street virus (*virus*

de rue) to that obtained from the nervous tissue of dogs in which the disease develops spontaneously. When the street virus is injected subdurally into the rabbit the latter develops hydrophobia only after an incubation period of from two to three weeks. If, however, this virus is passed from one rabbit to another, its virulence gradually increases until the incubation period decreases to six days.

Street Virus and Fixed Virus.

At this point it is called fixed virus (*virus fixe*), and its virulence can not be further increased. Passage through the cat, fox and wolf also increases virulence. On the other hand, by passing it repeatedly through the monkey (Pasteur), the chicken (Kraus) or the dog it becomes attenuated for the rabbit and virulence may be lost entirely.

Low Virulence of Fixed Virus for Man.

Although *virus fixe* represents its highest degree of virulence for rabbits, there is good reason for believing that repeated passage through the rabbit decreases the virulence of the virus for man. In other words, street virus is more infectious for man than fixed virus. This may to some extent account for the success of the Pasteur treatment. Ferran, indeed, uses unaltered *virus fixe* for the protective inoculation of man.

Distribution of Virus in the Body.

By means of inoculation experiments the virus may be demonstrated invariably in the brain, spinal cord, and usually in the salivary glands and saliva of animals which have died of the disease. These tissues are specifically affected, and the virus probably proliferates in them. By one or another observer its presence in the following organs and excretions has been demonstrated: Suprarenal gland, lachrymal gland, vitreous humor, urine, testicular secretion, lymph, milk, in the peripheral nerves and cerbrospinal fluid. Marx states that

it has not been found in the liver, spleen, blood and aqueous humor. Courmont and Nicolas found it, however, in the aqueous humor of rabbits after death. The possibility of postmortem invasion of this fluid has been suggested. It has been found occasionally in human saliva during life, and at the site of the wound following death (Pace).

Means of Infection.

Hydrophobia is transmitted almost exclusively by the bites of infected animals, the virus being conveyed in the saliva. Accidental inoculation may occur in handling infected tissues. The virus does not penetrate the intact skin, and it is customary to consider a bite as harmless unless the continuity of the skin is broken. Experimentally, infection has been caused by placing the virus on the mucous membranes of the conjunctiva, nose and mouth, in the absence of discernible lesions. Pace mentions a man who contracted the disease after his rabid dog had inserted the tip of its tongue in his (the patient's) nose. But one authentic example of transmission from man to man is found in medical literature. This occurred through kissing or biting, during coitus. In rare instances it seems to have been transmitted from the mother to the fetus in rabbits.

The dog is the most common carrier of hydrophobia. In some countries (Russia, Hungary) rabid wolves cause many infections. The disease has been conveyed by the bite of the cat, mouse and horse, and possibly by the skunk in some of our western states. The dog is, however, the natural host of the parasite, and either by his bite or by experimental inoculation practically all animals, at least mammalians, may be infected.

The incubation period in animals varies from

two weeks to several months. In man it varies between twenty and sixty days usually, but may be as short as seven or ten days, or as long as twenty months (rare). In children it is shorter than in adults. The location of the bite is also of importance in determining the length of incubation. It is shortest following wounds of the head and neck, somewhat longer when the injury is in the hand or arm, and still longer when in other parts of the body. The degree of laceration is also a factor, depending possibly on the introduction of larger quantities of virus, and on larger surfaces for its absorption. The bite of the wolf is said to be most virulent, and next in virulence is the bite of the cat and dog.

Incubation Period.

Not all who are bitten by rabid animals develop hydrophobia. Correct figures on this point are difficult to obtain, since in many instances the animals are only suspected of being rabid. According to Högyes, 15 to 16 per cent. of those who are bitten contract hydrophobia. The percentage is much higher following bites by the wolf. The disease is invariably fatal to man.

The symptoms of hydrophobia in man differ in no essential respects from those seen in animals.

Diagnosis in Dogs.

The immediate determination of hydrophobia in dogs which have bitten man is of the greatest importance. In many instances the behavior of the animal is sufficiently characteristic to justify clinical diagnosis of the disease. The disposition of the animal changes suddenly, it ceases to play, eats various indigestible substances, as glass, iron and wood, utters pathognomonic (?) long-drawn-out howls, may become ferocious, or, on the other hand, quiet and sullen. At autopsy the meninges

and nervous tissue are congested if the disease is advanced, and the indigestible mentioned substances may be found in the stomach, although the latter finding has little or no diagnostic importance.

So-called Specific Lesions.

A number of histologic changes have been described as characteristic. Among these are the bodies of Negri, described above. Remlinger attaches a great deal of importance to them as a means of diagnosis. Babès describes perivascular nodules of lymphoid cells (Wutknotchen) in the medulla and cord. The lesion of Van Gehuchten consists of a proliferation of the endothelial cells (neuronophages) surrounding the ganglionic cells, the latter at the same time undergoing atrophic and degenerative changes. This change is most marked in the cervical ganglia. One group of observers finds these lesions constant in animals which have died of hydrophobia, but they may be absent if the animal is killed during the course of the disease; hence their absence does not exclude the diagnosis of hydrophobia. Others have found similar changes in other diseases. Metchnikoff, it will be remembered, observed the destruction of ganglionic cells, by neuronophages in aged dogs (page 179).

We are hardly able at present to consider these changes as pathognomonic. Particularly in early stages of the disease they may be absent. The bite of a rabid dog is infectious in from two to four days in advance of the development of symptoms, and autopsy performed at this time may show neither gross nor microscopic changes which are characteristic. In communities in which hydrophobia is known to be endemic, all cases of dog bite accompanied by penetration of the skin should receive the Pasteur treatment.

Extension Through Nerves.

A great deal of experimental work which can not be given in detail shows conclusively that the virus is conveyed to the central nervous system by means of the peripheral nerves. The conditions then are similar to those in tetanus with this exception: In hydrophobia the living virus reaches the central nervous system, whereas in tetanus the bacilli remain at the site of the wound. This condition explains the shorter incubation period in hydrophobia, as in tetanus, when the infection atrium is near the central nervous system (e. g., face). When the infection is introduced into any particular part of the body surface, the virus is first demonstrable in the corresponding segment of the central nervous system. Although transmission by the nerves is the rule, infection may be accomplished in rabbits by intravascular injection. On the whole, however, infection is closely associated with the wounding of nerves. It has indeed been shown that if wounding of nerves is entirely avoided, as in intraperitoneal injections into rabbits (Marx) the full virulent nervous tissue may be used for immunization. A single injection of a large quantity brought about immunity in twelve days.

Prophylaxis.

The muzzling of dogs is a general prophylactic measure, which should be enforced in communities in which hydrophobia is known to occur. No matter how thoroughly the cauterization and antiseptic treatment of wounds is carried out it can in no case be depended on to destroy the virus. Even within five minutes the virus may be carried to a point which is beyond the reach of the cautery. In spite of this fact, however, cauterization should not be neglected, even when the Pasteur treatment

can be instituted at once. The greater the quantity of virus introduced by the bite the shorter will be the incubation period, and there is good reason to believe that cauterization (actual cautery) properly carried out destroys a sufficient amount of virus to prolong the incubation period. A long incubation period is greatly in favor of the success of the Pasteur treatment.

Preparation of Virus for Pasteur Treatment.

Pasteur's first protective inoculations were carried out with virus which had been attenuated by passage through the monkey. The *virus fixe* obtained from the rabbit, as described above, was soon substituted for that of the monkey. In order that an antirabic institute may continuously have on hand a sufficient amount of vaccine, it is necessary to inoculate two or three rabbits daily. For this purpose an emulsion of the medulla of a rabbit which has died of hydrophobia is inoculated beneath the dura mater. A short time before the animals would die of the disease, they are killed by bleeding, and the spinal cords removed with all possible precautions for asepsis. Each cord is cut into two parts and each part suspended in a properly constructed jar which contains solid potassium hydrate. After the jar is sealed desiccation is allowed to proceed for fourteen days, at the end of which time the infectiousness of the tissue has so decreased that it is suitable for the first injection. The vaccine should be free from bacteria.

Technic of Treatment.

As is well known, the Pasteur prophylactic treatment consists of the subcutaneous injection on successive days, of suitable quantities of *virus fixe,* prepared as described above, beginning with the cord which has been desiccated for fourteen days and gradually using fresher cords until viru-

lent virus has been inoculated. The vaccine is prepared for use by emulsifying one centimeter of a cord in 5 c.c. of salt solution or some "artificial serum," and in a single treatment from 1 to 3 c.c. of this emulsion is injected, usually into the subcutaneous tissue of the anterior abdominal wall. In this region there is less likelihood of injuring large nerves, and local complications, which, however, occur rarely, are of less consequence.

The rapidity with which one should pass from the fourteen-day cord to fresh virus depends on the urgency of the case. When there is good reason to suspect a short incubation period, or when some days have followed the bite an "intensive" treatment should be used; in other cases the progression may be slower. The following conditions augur a short incubation period: Bites of children, who are more susceptible than adults, and in whom the injuries usually are on the face; bites on the face and neck in all cases; lacerated wounds in which there is a larger surface for absorption of the virus. The influence which proper cauterization exerts on the incubation period was mentioned above.

The table on page 520, taken from Marx, illustrates a "light" and an "intensive" treatment.

This scheme is variously modified in different institutes, especially in the direction of a more rapid progression to virulent material.

Other Means of Attenuation.

Other methods of attentuation are also used, as the following: Heating emulsions of fresh virus at 58° C. for different lengths of time, or at different temperatures (80° to 30° C.) for ten minutes (Babès-Puscari); digestion of virus with natural or artificial gastric juice (Tizzoni and Cen-

tanni); the use of fresh but very dilute virus (Högyes). Ferran, in Barcelona, inoculates man with the fresh unaltered *virus fixe,* and in nearly 2,000 cases but two cases of hydrophobia developed. This indicates clearly the low infectiousness of *virus fixe* for man.

Light.			Intensive.		
Day of Treatment.	Age of Dried Cord in Days.	Amount of Emulsion Injected.	Day of Treatment.	Age of Dried Cord in Days.	Amount of Emulsion Injected.
1	14	3	1	14	3
	13	3		13	3
2	12	3		12	3
	11	3		11	3
3	10	3	2	10	3
	9	3		9	3
4	8	3		8	3
	7	3		7	3
5	6	2	3	6	2
	6	2		6	2
6	5	2	4	5	2
7	5	2	5	5	2
8	4	2	6	4	2
9	3	1	7	3	1
10	5	2	8	4	2
11	5	2	9	3	1
12	4	2	10	5	2
13	4	2	11	5	2
14	3	2	12	4	2
15	3	2	13	4	2
16	5	2	14	3	2
17	4	2	15	3	2
18	3	2	16	5	2
			17	4	2
			18	3	2
			19	5	2
			20	4	2
			21	3	2

It seems unnecessary at this date to quote statistics to show the value of the Pasteur treatment. Observations indicate that immunity is not fully established until about fourteen days after the completion of the treatment, and in a certain number of cases the disease develops before this time has passed. The number of deaths after this period is exceedingly small and has grown less with

improved technic. In 1886 the number of deaths which occurred after fifteen days had passed amounted to 0.94 per cent.; in 1902 to 0.18 per cent.

Immunity and Serum Properties.

The immunity established by the Pasteur treatment is, in all probability, antimicrobic in nature. The serum of both man and animals, after immunization, is able to destroy the infectiousness of rabic nervous tissue, i. e., the serum is rabicidal (Babès and Lepp, 1889). The technic of Kraus and his co-laborers is well adapted to show the rabicidal properties of the immune serum. Rabid nervous tissue is made into an emulsion with salt solution in a dilution of 1-100, and then filtered through paper to remove coarse particles of tissue. To quantities of 0.5 to 1.0 c.c. of this emulsion varying amounts of fresh immune serum are added, and after eighteen hours' contact the mixtures are injected into rabbits to determine the degree of infectiousness. Small quantities of rabicidal substance may be detected in this way.

Natural resistance to hydrophobia does not go hand in hand with the antirabic power of an animal's serum. Old pigeons, for example, develop the disease following intracerebral injection of the virus, although their serum is not rabicidal.

Babès and Lepp also showed that the immune serum has protective powers which are analogous in their efficiency with those of bactericidal serums. Babès advocates and practices the mixed method of immunization in severe cases, immune serum being injected in addition to the virus. The serum has little or no curative value.

II. SYPHILIS.

Hypothetical Causes.

It is impossible in this place to describe or even mention the many cocci, bacteria and protozoa (?) which have been brought into etiologic relationship with syphilis. Until very recent times the bacillus of Lustgarten (*Bacillus syphilis* (?)), occupied a fairly prominent position as the possible cause. This organism resembles the tubercle bacillus in its morphology and staining properties, and is not to be differentiated from one of the smegma bacilli. Its recognition in syphilitic lesions has always been difficult, and by far the greatest number of investigators have been unable to demonstrate it. It has never received general recognition as the cause of the disease, and its presence in lesions of the genitals has no significance because of the occurrence of smegma bacilli in this locality.

The bacillus of De Lisle and Julien, and that of Joseph and Piorkowski rest on no better basis.

Two important discoveries of recent date lend to the hope that some light may be thrown on many dark problems in relation to syphilis.

Transmission to Monkeys.

The first has to do with the transmission of the disease to lower animals. Such attempts have been very numerous, both by the inoculation of syphilitic tissues and of organisms cultivated from the tissues. We have not the space to describe individual experiments, and can only say that transmission to various animals (monkey, guinea-pig, rabbit, hog, etc.) has been claimed in a number of instances.

Leaving the monkey out of consideration, it is reasonably certain that none of these reported successes represented the production of syphilis; this is shown decisively by experiments published

by Neisser in 1902. This may not be true, however, in regard to the inoculation of monkeys, in which successful experiments were reported by Klebs (1879), Martineau and Hamonic (1882) and Sperk (1886-8).* It is quite likely that Sperk, in particular, accomplished transmission several times. However, the successes were not uniform, and the possibility of such transmission has become an assured fact only in the most recent times.

Experiments of Metchnikoff and Roux.

It occurred to Metchnikoff and Roux as it had occurred to others that the monkey, particularly the higher species (chimpanzees), should on account of their biologic proximity to man, be the most suitable animal for the production of experimental syphilis. Attention has already been called to this proximity as indicated by the reaction of serum precipitins.

Transmission from Monkey to Monkey.

Their first inoculation was performed on a female chimpanzee, virus from a primary lesion and from mucous patches being introduced by means of scarification into the prepuce of the clitoris and into the skin of the eyebrow. The wounds healed, and twenty-six days after inoculation a vesicle which soon was surrounded by induration appeared on the prepuce. This lesion was pronounced a typical hard chancre by eminent dermatologists and syphilologists. With the appearance of the chancre the inguinal lymph glands became enlarged and one month later a papular eruption appeared on the thighs, abdomen and back. The papules persisted for more than a month, and were still discernible when the animal died several weeks later of pneumococcus infection. Before this ani-

* Flexner: "The Etiology of Syphilis," Med. News, Dec. 9, 1905.

mal died a second chimpanzee was inoculated from the primary and secondary lesions of the first animal, resulting in the development of primary lesions and of adenitis. Still another successful inoculation resulted in secondary lesions with the formation of mucous plaques. They have since performed many similar experiments with positive results, when the higher types of monkeys were used. Confirmation has come from a number of independent experimenters (e. g., Lassar, A. Neisser, Kraus, Flexner), and A. Neisser in particular has taken up the work on an extensive scale.

Experiments of Neisser.

Some of Neisser's work is of the utmost importance. The experiments of Metchnikoff and Roux had already indicated that the higher monkeys (chimpanzee, etc.) acquired generalized syphilis more readily than the lower species. Neisser's work corroborates this, and he recognizes a scale of susceptibility which corresponds roughly with the proximity of the different species to man, as indicated by general morphology and the reaction of serum precipitins. The chimpanzee, orang-utan and gorilla are the most susceptible, and the syphilis produced in them approaches closely that seen in man, including the secondary symptoms. It is suspected that the cynocephalus varieties are less, and the macacus varieties least susceptible. Among the macaci the smaller types (rhesus) are more resistant than the larger. The lower susceptibility of these animals is recognized by the failure of secondary symptoms to develop, hence in them the syphilis may be purely local (Neisser). The study of experimental syphilis is so young that generalizations at this time are out of the question.

A second discovery of no less importance was

that of a spirocheta* (*Spirocheta pallida*) in the primary and secondary lesions of syphilis by Hoffmann and Schaudinn in 1905. "The organism measures from 4 to 10 microns in length, the average being about that of the erythrocyte of man. Its width varies from immeasurable thinness to one-half micron. It possesses from three to twelve, sometimes more, curves, which are sharp and regular and resemble the curves of a corkscrew. The poles are sharpened. The organism is mobile, and the motions consist of rotations on the long axis, forward and backward movements, and the bending of the entire body. Flagella have not been seen" (Flexner). It may be stained by the azur of Giemsa or the Romanowsky stain or some one of its modifications. Confirmation has come from a large number of observers in rapid succession, little difficulty being found in demonstrating the spirals in preparations from chancres and mucous plaques, secondary lesions in the skin and in fluid aspirated from the lymph glands which are regional to the chancre. Schaudinn found it once in fluid aspirated from the spleen, but its demonstration in the blood has been accomplished in only a few instances after prolonged centrifugation of the blood. Levaditi and Petresco discovered it in the fluid of an artificially produced blister. Two additional facts make the case of *Spirocheta pallida* a strong one, i. e., its occurrence in the lesions of the congenitally syphilitic and in the experimental lesions of the monkey, even when the inoc-

Spirocheta Pallida, the Possible Cause.

Distribution of Spirocheta.

* The discovery of spirochetæ in yaws, by Castellani and by Wellman, is a very suggestive one, in view of the tendency in many quarters to considers yaws as a manifestation of syphilis.

ulation is made from a previously infected monkey (Kraus). Knowledge concerning its relation to tertiary lesions is not yet of a satisfactory nature.

Suggestive as these results are, we must appreciate that much remains to be learned before the causal relationship of *Spirocheta pallida* to syphilis can be an unquestioned one.

Infection. Infection usually is venereal. It is not definitely known whether a defect of the surface of the prepuce, glans, vagina, etc., is essential for infection. The epithelium in these localities is so delicate that defects of microscopic dimensions may be easily produced, and infection may take place through such defects as through grosser lesions. It is well known that the lip, tongue, conjunctiva and finger may be the seats of primary lesions, and it is probable that no part of the body surface is immune when the virus is introduced suitably.

Occurrence of Virus. The secretions of all surface lesions in syphilis are infectious, except those of gummata. Concerning the infectiousness of different normal secretions and the situation of the virus in internal organs, we may look forward to more positive information than we have had hitherto. Defibrinated blood and serum from cases of secondary syphilis did not produce lesions when injected subcutaneously and intraperitoneally into monkeys (Neisser). Neisser had previously performed experiments on man which showed the serum not to be infectious. We can not conclude from these results, however, that the blood in syphilis is not infected. Inoculations into monkeys from the internal organs (liver, spleen, bone marrow) of syphilitic monkeys, gave negative results. The virus is nonfilterable, i. e., it is so large or its form is such that it does not pass through a Berkefeld filter.

Virulence.

Clinical experience indicates that the virulence of the syphilitic virus is not uniform. It is possible that certain strains are more likely to bring about "post-syphilitic" diseases than others. That the resistance of the virus outside the body is low seems evident from the fact that transmission is practically unknown except as it occurs by direct contact. Neisser destroyed it by heating to 60° C. for thirty minutes, but at this temperature for ten to twenty minutes its virulence for monkeys was retained.

Prophylaxis demands no principles not generally known.

Susceptibility to syphilis varies a great deal, not in the sense that some are immune, but in that a more virulent type of disease develops in some than in others. This is a condition, however, which is difficult to differentiate from variations in the virulence of the infecting agent. Syphilis is said to be particularly virulent when introduced into a race of people for the first time.

Immunity.

The subject of immunity to syphilis is one of such proportions, the phenomena are so varied, and knowledge so inaccurate, that a thorough analysis can not be undertaken in this place. It is the customary belief that one attack confers permanent immunity to a second. To what extent the acquired resistance to reinfection signifies a state of immunity is not satisfactorily settled. It seems well established that within a relatively short period following the appearance of a chancre a second primary lesion can not be acquired. It would be impossible to refer this resistance to actual immunity in view of the fact that the individual is at the moment the subject of systemic infection. A second infection would be but a su-

perimposed infection and may not be recognizable without the formation of a new chancre. The resistance which is continued into the tertiary stage, at a time when the individual usually has lost infectiousness for others, is equally obscure. If the present hope that *Spirocheta pallida* will be shown to be the cause of the disease is realized, and if experimental work with the monkey yields the results which it seems to promise, these and many other questions of fundamental importance may be elucidated. Among such questions are: the immunity of a mother who gives birth to a child infected by the father at the time of conception (Colle's law); the occasional occurrence of reinfection; the persistence of infectiousness and transmission of the disease into the third generation, of which there are a number of reported examples.

Serum Therapy.

Serum-therapy, or vaccination against syphilis, are possibilities of a future time. A truly antisyphilitic serum has not yet been demonstrated. Neisser found the serum of syphilitics, from whatsoever stage of the disease, without influence on the course of the infection. Also treatment with the normal serums of various insusceptible animals has had no unqualified success. Metchnikoff and Roux observed a phenomenon which suggested to them the possibility of attenuating the virus so that it may be suitable for vaccination. A macacus monkey reacted to inoculation by the production of a local lesion, the virus of which when transferred to the more susceptible chimpanzee likewise caused a local lesion, but no signs of generalization appeared. The chimpanzee later showed himself resistant to the virus from man, and for

this reason it was assumed that immunization had been accomplished. Neisser reasonably criticises this conclusion on the ground that the chimpanzee, having been inoculated with syphilis from the monkey, resisted inoculation from man, not because he was immunized, but because he was syphilized, i. e., he was already infected with syphilis.

III. YELLOW FEVER.

Occurrence.

Yellow fever is peculiarly an American disease, and it has reached other continents (e. g., Spain) only in accidental ways and for brief periods. It is possibly endemic in certain portions of West Africa (Sierra Leone), to which it was probably carried from the Antilles (Scheube). Scheube regards the Antilles as the birthplace of yellow fever. Knowledge of it extends only to the middle of the seventeenth century, at which time it surely existed in the West Indies. The disease has on several occasions been carried to Spain by vessels returning from Cuban ports. Until very recent times it was endemic in Cuba, especially Havana, and in Vera Cruz and other Spanish-American ports it has prevailed extensively. From such points extension frequently takes place into adjacent tropical or subtropical regions, or even into temperate localities during the summer months. In the latter part of the eighteenth century Philadelphia suffered very severely. Baltimore was attacked similarly and Boston to a less degree. Other northern ports, e. g., New York, have experienced attacks of limited duration, the disease, presumably, being introduced by means of infected ships.

In addition to our southern coasts and that of Mexico, the Atlantic coast of South America has

been infected as far south as Buenos Ayres, and likewise the western coast of Mexico and Peru. In the eighteenth century the coast of Spain and Portugal suffered severely, but since that time only minor epidemics have occurred in these countries. Epidemics frequently have appeared on ships after they had left infected ports.

The Southern States were invaded repeatedly in the last decade of the eighteenth century, in 1803, 1805, 1853, 1867, 1873, 1878, 1905, and in lesser degrees at other times, in all ninety-six times. The severest epidemics were those of 1853 and 1878.

Bacillus Icteroides.

The many microbes which have been cited as the cause of yellow fever need not be described. The *Bacillus icteroides* of Sanarelli, which had attained more prominence than any other, was shown by Sternberg, by Reed and Carroll and by the more recent work on the mosquito theory, to bear no causal relationship to the disease. According to Reed and Carroll it is identical with the hog-cholera bacillus.

The monumental work of Reed, Carroll, Agramonte and Lazear (1900), the last of whom lost his life from yellow fever, has made it possible to replace accurate knowledge of the epidemiology and prophylaxis of yellow fever and, to a certain extent, of its etiology, for many incorrect ideas which had prevailed up to that time.

The Mosquito Theory.

The conception that yellow fever is transferred from one person to another by mosquitoes was first advanced positively by Carlos Finlay, a Cuban physician, in 1881, although several American physicians had long before noted the prevalence of mosquitoes during yellow fever outbreaks (Rush, 1793; Weightman, 1839; Wood, 1853;

Barton, 1853). He reported the transmission of the disease, experimentally, by the bites of mosquitoes which had fed on yellow fever patients, and stated that light attacks which followed the bites resulted in the establishment of immunity. The subsequent observations of Reed and his co-workers indicate, however, that Finlay's technic was such that he could not possibly have produced experimental fever, and that the development of the disease in his subjects was purely a coincidence. The reason for this will appear below.

The Work of Reed, Carroll, Etc., with Stegomyia Fasciata.

Having satisfied themselves that *Bacillus icteroides* is but an accidental organism in yellow fever, and that it is found under normal conditions as well, Reed and his associates began work on the mosquito hypothesis of Finlay. The first positive result was obtained in the case of Dr. Carroll. Carroll "was bitten at 2 p. m., Aug. 27, 1900, by *Stegomyia fasciata.* This particular mosquito had bitten a severe case of yellow fever on the second day of the disease, twelve days before; a mild case of yellow fever on the first day of the attack, six days preceding; a severe case of yellow fever on the second day of the attack, four days before; a mild case of yellow fever on the second day of attack, two days before inoculation." After an incubation period of three days, Carroll developed typical and severe yellow fever, from which he recovered. A similar result in one other case was reported at this time, and later Camp Lazear, with mosquito-proof houses, was established for the continuation of the study. The experiments of Reed and his co-workers, and confirmatory work by Guiteras and the French commission, can not be described in this place. We may feel sure, how-

Important Facts Which Have Been Learned.

ever, that with all the conditions of experimentation under absolute control the following points have been determined with scientific certainty: 1. Yellow fever may be transferred from a patient to a non-immune by the bite of a mosquito—*Stegomyia fasciata*—which has previously fed on the yellow fever patient. 2. In order that the mosquito become infected it is necessary for him to feed on yellow fever blood within the first few days (three days) of the fever. 3. The mosquito can not transfer yellow fever directly and immediately from the patient to a non-immune, but it is necessary for a period of not less than twelve days to elapse before he becomes infectious. When this time has been reached the insect continues infectious for at least fifty-seven days and probably throughout his life. 4. Yellow fever can not be transferred by "fomites." 5. The subcutaneous injection of yellow fever blood into a non-immune produces yellow fever, hence the infecting agent exists in the circulation. 6. The serum of a yellow fever patient, after being diluted and filtered through a Berkefeld filter (Reed and Carroll) or Chamberland B porcelain filter (Rosenau, Parker, Francis and Beyer) is infectious, hence the infecting agent at some stage of its development is very minute, possibly ultramicroscopic. 7. "An attack of yellow fever produced by the bite of a mosquito confers immunity against the subsequent injection of the blood of an individual suffering from the non-experimental form of this disease" (Reed, Carroll and Agramonte). 8. The period of incubation usually is three days, but may vary within the limits of two to six days. 9. "A house may be said to be infected with yellow fever only

when there are present within its walls contaminated mosquitoes capable of conveying the parasite of the disease." 10. "The spread of yellow fever can be most effectually controlled by measures directed to the destruction of mosquitoes and the protection of the sick against the bites of these insects." 11. No mosquito other than *Stegomyia fasciata* has been found capable of transmitting the disease, and analogies suggest the probability that no other insect is concerned.

Epidemiology and Stegomyia.

These discoveries explain many facts in relation to yellow fever which had been obscure hitherto. For example, yellow fever is a tropical and subtropical disease only because *Stegomyia fasciata* breeds in tropical and subtropical climates. The disease is found in low, moist localities rather than in the high and dry, because the mosquito inhabits the former and not the latter. Yellow fever dies out with the first severe frost or on the advent of cool weather because these conditions either kill the mosquito or cause him to hibernate. The advent of an initial case of yellow fever in a suitable region is followed by the appearance of the disease in epidemic form only after a period of two or three weeks, because the mosquito first becomes infectious in about two weeks after it has fed on yellow fever blood; this may correspond with a certain stage of development of the as yet unrecognized parasite. The observation often made that yellow fever, like malaria, is not contagious in the ordinary sense, in spite of its rapid extension, is readily understood, as is the irregular method in wihch the disease spreads. It is now clear why the disinfection of fomites has never been able to check the advance of an epidemic, and why the

ordinary quarantine measures which did not take the mosquito into consideration were not effective in keeping the disease out of a favorable port; and by a favorable port is meant one which can harbor *Stegomyia fasciata*. These discoveries also explain how yellow fever could be stamped out of Havana, Texas and New Orleans by prophylactic, hygienic and quarantine measures, which had as their objects the destruction of the mosquito and its breeding places and prevention of the infection of the mosquitoes by suitably screening the patients.

Distribution of Stegomyia.

It is thus seen that the epidemic occurrence of yellow fever is strictly associated with the distribution of *Stegomyia fasciata*. Howard, in Bulletin No. 46 of the Public Health Reports, gives this distribution as known on Sept. 10, 1905, and publishes a map showing the region which the insect may be expected to inhabit.

Stegomyia fasciata has been found in the following localities in the United States (Howard):

Virginia: Virginia Beach, Norfolk, Lynchburg, Danville, Richmond. *Kentucky:* Lexington, Middlesboro, Louisville, Richmond. *Illinois:* Cairo. *Tennessee:* Nashville, Knoxville, Clarksville, Chattanooga, Memphis, Columbia, Decherd, Athens, Bristol. *Arkansas:* Hot Springs, Helena. *Louisiana:* Ruddock, New Orleans, Baton Rouge, Napoleonville, Covington, Hammond, Shreveport, Franklin, Morgan City, New Iberia, Patterson. *Mississippi:* Pass Christian, Summit, Quarantine Station, Vicksburg, Clarksdale, Tutwiler, Belzoni, Holly Springs, Jackson, Wonona, West Point, Tupelo, Corinth, Agricultural College, Biloxi. *Alabama:* Mobile, Decatur, Auburn, Tuscumbia, Huntsville, Yazoo City. *Georgia:* Atlanta, Pelham, Augusta, Savannah, Brunswick. *Florida:* Barrancas, Key West. *Texas:* Galveston, Houston, Victoria, San Diego, Tyler, Laredo, Austin, San Antonio, Corsicana, Brownsville, Alice, Colorado, Dallas, Paris, Edna, Fort Bliss (El Paso), Fort Ring-

gold (Rio Grande-Ludlow). *South Carolina:* Charleston, Columbia, Fort Fremont, Sullivan's Island. *Arizona:* Nogales. *Maryland:* Baltimore (Carter)—breeding in fresh water on fruit wharf. *North Carolina:* Beaufort, Winston, Raleigh, Greensboro, Charlotte, Salisbury. *Indiana:* Jeffersonville. *Missouri:* St. Louis.

Breeding Places and Life Cycle.

Reed and Carroll found the larvæ of stegomyia "(1) in rain-water barrels; (2) in tin cans that had been used for removing excreta and which still contained a small amount of fecal matter; (3) in sagging gutters containing rain water; (4) in cesspools; (5) in tin cans placed about table legs to prevent the inroads of red ants; (6) in the collection of water at the base of the leaves of the *agave americana;* (7) in one end of a horse trough that was in daily use." These instances are cited to show the general character of the places in which the eggs and larvæ of stegomyia may be found. The eggs are deposited during the night, in about seven days after the ingestion of blood, and "in pairs, in groups of three or more or singly," to the number of forty-seven on the average (Reed and Carroll). The eggs are very resistant to drying and extreme cold (—17° C.). With a favorable temperature they hatch in from three to seven days; the larval stage lasts for seven days, the pupal two days, the total cycle being completed in about twelve days. As in the case of anopheles, only the female stegomyia sucks blood. The insect prefers the hours from 3 p. m. to 9 a. m. for feeding, but is most active from 4 p. m. to midnight. "In captivity the hungry impregnated female will bite at any hour of the day or night." In a state of freedom it will not bite a second time for from five to seven days. It appears not to bite when the temperature is lower

Time of Biting.

than 62° F., another factor in the subsidence of yellow fever with the advent of cool weather. For further details concerning the morphology, biology and habits of stegomyia consult Howard on "The Mosquito"; Reed and Carroll, "The Prevention of Yellow Fever," *Medical Record,* Oct. 26, 1901; Parker, Beyer and Pothier, "Report of Working Party No. 1," Yellow Fever Institute Bulletin No. 13, 1903, Washington.

Importation by Ships.

Yellow fever cases and stegomyia work together in the extension of the disease just as malarial cases and anopheles do in the extension of malaria; for the principles involved the chapter on malaria may be consulted. Of particular interest is the importation of the disease by means of ships, since the invasion of the United States usually comes about in this way. It is frequently stated that ships lying one-half mile from shore are safe from yellow fever; Grubbs, however, believes that stegomyia may reach vessels lying within fifteen miles of the shore if the wind is favorable. The insect readily boards a vessel lying in an infected port and may remain there at least during a seventeen days' voyage. It may also breed in suitable barrels or tanks of water on the ship. Under these conditions it is readily understood how a ship, leaving a harbor with a healthy crew, may be attacked by yellow fever a few days after leaving port; and how any quarantine measure at a new port which does not involve the destruction of the mosquitoes on the boat and the protection of the patients from the bites of mosquitoes is inadequate.

Resistance of Virus.

As stated, the nature of the virus is unknown. Its filterability was mentioned. A temperature of 55° C. for ten minutes renders innocuous

the defibrinated blood of the infected; according to the French Commission (Marchoux, Salimbeni and Simond) the virus is destroyed in five minutes at this temperature. The latter also found that defibrinated blood when sealed under vaselin retained its virulence for five, but not for eight days. The toxic substance appears to have a strong affinity for the parenchymatous organs, particularly the liver and kidney.

Prophylaxis.

The essential principles of prophylaxis have been alluded to: 1, the destruction of breeding places for the mosquito as described in the section on malaria; 2, the isolation of patients, screened, to exclude mosquitoes; 3, the destruction of mosquitoes found in infected houses or ships; 4, the individual factor of avoiding the bites of mosquitoes, which involves the screening of houses, and individual care. One may go about more safely in the middle of the day than before 9 a. m. and after 3 p. m. For the disinfection of houses, i. e., for the destruction of mosquitoes, two pounds of tobacco or two pounds of pyrethrum powder per 1,000 cubic feet of space may be burned after the rooms are sealed. When smaller quantities are used the insects may be only stupified, and should be collected and burned (Rosenau, Parker, Beyer and Pothier). Sulphur dioxid is highly efficient, but formaldehyd is valueless as an insecticide (Rosenau).

Susceptibility.

The negro is less susceptible to yellow fever than the white man and in him the mortality is lower. Among the natives the mortality is from 7 to 10 per cent., among the whites from 20 to 80 per cent. (Scheube). The statement that Caucasians may become "acclimated" so that they are less suscep-

YALE MEDICAL LIBRARY

tible needs additional investigation. It seems impossible that acclimatization could mean anything else than active immunization. Children and the aged are attacked less frequently than those between the ages of ten and thirty.

Immunity and Serum Properties.

An attack of yellow fever, whether experimental or natural, confers immunity of long or lasting duration. According to the French Commission, a certain degree of immunity could be conferred by the injection of infected serum which had been heated to 55° C. for five minutes, or of defibrinated blood which had been kept under vaselin oil at room temperature for eight days. They also claimed that the serum of convalescents has prophylactic and curative properties to a certain degree.

IV. TYPHUS FEVER.

In addition to a streptobacillus obtained by Hlawa and the diplococci described by a number of investigators, a supposed protozoon, resembling pyroplasma, was found in six cases by Gotschlich. The etiologic rôle of none of these organisms can be accepted at the present time.

Occurrence and Contagiousness.

Typhus is now a rare disease. It is endemic on a small scale in London, Glasgow and Liverpool, and cases occur in the larger cities of Ireland. In epidemic form it attacks localities in which the hygienic conditions are bad. The contagion seems to fasten itself in such localities and does not extend with rapidity to neighboring communities in which good hygiene and cleanliness prevail; it is particularly a disease of the poor, the filthy and the underfed. Healthy, clean and well-nourished persons who enter an infected district and come in contact with the patients are subject to

attack. Typhus has always been considered a very contagious disease. It has been noted repeatedly, however, that when patients are removed to a hospital and kept under clean and hygienic conditions with plenty of fresh air that infection of attendants and physicians is relatively infrequent. From observation of 600 hospital cases Robinson and Potts draw particular attention to this point and lay great stress on the importance of liberal ventilation in decreasing contagiousness. The method of transmission is not known. It has been suggested repeatedly that the disease may be spread by the bites of insects, perhaps fleas and bed-bugs. Gotschlich emphasizes the latter as possible carriers, discrediting the significance of the flea. He notes that in Alexandria fleas are everywhere, whereas typhus is confined largely to the poorer and unclean localities. Transmission by means of clothing and other fomites is said to occur. Usually the development of typhus in a hitherto uninfected community is traceable to the importation of the disease; in some instances, however, the origin could not be learned.

Prophylaxis demands the isolation and disinfection usually practiced in combating contagious diseases, particular attention being paid to hygiene, cleanliness, the admission of fresh air to the sick room, and the destruction of vermin (!).

The serum of convalescents is said to be curative in a moderate degree (Legrain).

V. DENGUE FEVER.

Occurrence.

Dengue occurs in numerous countries which afford a warm climate. It is endemic in Egypt, Arabia, Senegambia, Honduras, the Bermudas,

and the Sandwich Islands. Important centers for the origin of epidemics are the lesser Antilles of the Western Hemisphere, the Red Sea Coast, and Senegambia (de Brun, cited by Scheube). It occurs in our southern states and in Mexico. It may be introduced into new regions by means of infected ships.

Organisms. The specific agent is unknown. The "plasmeba" described by Eberle (1904) and his hypothesis that *Culex fatigans* may transmit the disease await confirmation. The same may be said of an influenza-like organism seen by Carpenter and Sutton (1905) in stained preparations from the throat.

"Dengue fever is an acute infectious disease, distinguished by the appearance of an initial and terminal polymorphous eruption and accompanied by severe articular and muscular pains." Corresponding with the two eruptions, there are characteristically two periods of temperature separated by a short period of apyrexia. The intense muscular pains and asthenia resemble those of influenza, the respiratory affections of the latter being absent, however. The incubation period varies from a few hours to four or five days, usually one or two, and the entire duration from six to seven days.

Transmission. Dengue fever extends epidemically with all the rapidity which characterizes influenza. "In some respects the spread of the disease suggests some peculiarity in the method of propagation differing from that of the well-known diseases, influenza, scarlet fever, measles, etc. It appeared to spread particularly to contiguous houses, whole streets being attacked seriatim."* Dengue prevails espe-

* "Report on the Dengue Epidemic in Brisbane in 1905." Committee of Queensland Branch of the British Medical Association. Journal of Tropical Medicine, Dec. 15, 1905.

cially during the hot months. "A great fall of temperature and the appearance of absolutely cold weather always puts an end to the epidemics" (Hirsch, cited by Scheube).

The disease extends so rapidly, and the incubation period is so short, that general measures of prophylaxis would seem to be of no avail.

Susceptibility is general, even in infancy and old age. The disease has a very low mortality; it is more severe in very early and very late life. Relapses are not infrequent, and one attack does not confer immunity. Second attacks may occur during the same year. Leucopenia is present from the first (Carpenter and Sutton).

VI. ACUTE ARTICULAR RHEUMATISM.

(See pp. 359, 360.)

VII. SMALLPOX AND VACCINIA.

Relation of Vaccinia to Smallpox.

Vaccinia and smallpox may be considered together, having in mind the likelihood or, indeed, the certainty, that they have a common etiology. This view seems the only possible one, in spite of our uncertainty as to the exact nature of the cause. To hold a different view would be to acknowledge that immunization with one kind of microbe may confer immunity of the strongest and most specific character against another, a condition for which we could find no parallel.

Inoculation of the Calf with Smallpox.

More satisfactory knowledge, however, comes from actual conversion of smallpox virus into vaccine virus by passing the former through cows. Abbot quotes W. J. Simpson as follows: "In November, 1885, with smallpox lymph from an unvaccinated patient, I inoculated a cow with fifth-day lymph and a ewe with eight-day lymph from

the same patient. Both presented vesicles on the seventh day, the lymph of which I sent to London to be used by Dr. Cory, the director of the Animal Vaccine Institute of London. This calf lymph, which Dr. Cory passed through a second calf before using it on children, was the starting point of a new vaccine at the institute. Between Nov. 21, 1885, and May 6, 1886, 1,247 children had been vaccinated with this lymph and gave 98.4 per cent. insertions of success."

Concerning the changes which smallpox virus undergoes in the cow, as a result of which it loses permanently the power of causing smallpox in man, we have no knowledge, aside from the hypothesis of Councilman and others mentioned below.

Etiology. We may pass over the various bacilli and cocci which have been described as causing vaccinia and smallpox with the remark that none of them are of primary significance, but that they have been either accidental contaminations or the causes of secondary infections during the course of the disease.

Theories. There are two chief theories as to the cause of smallpox (and vaccinia) to-day. One, that the virus is an ultra-microscopic and uncultivatable organism; and a second, that it is represented by certain protozoon-like bodies seen in the specific lesions (vesicles, pustules) of both vaccinia and smallpox. Concerning the first theory we know nothing beyond the observation of Parke that the virus of both vaccinia and variola did not pass through Berkefeld and Chamberland filters under the conditions of his experiments. Of the second theory a brief review may be given.

Protozoon-like bodies have been seen by many

observers and were first brought into causal relation with smallpox by Van der Loeff and by L. Pfieffer (1887). Guarnieri (1892), however, gave the subject its present impetus by a careful study of these forms as seen in vaccinia and gave to the hypothetical organism the name of *Cytoryctes vacciniæ, s. variolæ.* The bodies were found within the deep epithelial cells in the pustules of vaccinia and smallpox and in the lesions produced on the cornea of the rabbit by inoculation with the viruses of vaccinia and smallpox. They lie within clear spaces in the protoplasm of the cells, vary in size from that of a micrococcus to that of an epithelial nucleus and multiply, it was supposed, by binary division. When mounted in hanging-drops of the vesicular fluid they showed ameboid movements. Confirmatory work came from others, and particularly Wasielewski, who concluded that the "vaccine bodies" are perfectly characteristic, that they are never found in normal or other pathological conditions of the skin, that they can not originate from leucocytes or epithelial cells, and hence can not be accidental "cell inclusions." Filtered virus produced no lesions in the cornea of the rabbit.

Cytoryctes Variolæ, a Protozoon(?)

Recently Councilman, Magrath and Brinckerhoff have studied this supposed organism in great detail and find, in addition to the forms in the cytoplasm (cytoplasmic parasites), still others within the nucleus of the epithelial cells of the vesicles and pustules. They express the belief that the organism first gains entrance in the cytoplasm of the cells, and after a period of "multiplicative proliferation," the products of the latter process penetrate the nuclei of the epithelial cells and there undergo another type of proliferation. Calkins,

Work of Councilman and Others.

the zoologist, after studying the material, shares their views and has constructed a life cycle of the parasite from the various forms which he found in fixed and stained preparations.

Life History of Cytoryctes.

The smallest recognizable forms in the cytoplasm measure about 0.7 of a micron and lie in a vacuole in the cytoplasm near the nucleus. Calkins interprets these as "gemmules" and as products of the proliferation of the parasite at the primary point of infection (lungs (?)). Somewhat larger forms (3 microns) containing a vacuole with a central point staining with methylene blue, represent "gemmules" which have grown and have become somewhat differentiated. The periphery

Cytoplasmic Stages.

of the organism becomes differentiated also by the formation of minute dots which may eventually be stained by a special method. During this stage the organism "often is spherical, but may be fusiform, pyriform or ameboid, while pseudopodia are frequently caught in various degrees of extension." No definite nucleus is discernible, but material corresponding to nuclear substance is distributed somewhat generally through the parasitic cell. Certain granules are distributed throughout the body of the organism, and these granules eventually give rise to the "gemmules" or young parasites which become free by the disintegration of the mother cell.

Howard and Perkins find, in addition to the cytoplasmic stage of Councilman and his co-workers, a second cytoplasmic stage, the products of which penetrate the nucleus to institute the intranuclear stages. Calkins speaks of the fate of the gemmules as follows: "The germs formed by the multiplicative reproduction of the cytoplasmic ameboid form of the parasite may develop into new cytoplasmic organisms or ultimately may become germ cells within the nucleus of the epithelial cell. In the latter case they develop into structures which I regard as gametocytes. The resulting zygote (formed by conjugation of the gametes) is the ameboid pansporoblast mother organism."

Nuclear Stages.

The conclusion that conjugation takes place is based on certain analogies with other micro-organisms, rather than on observation of the phenomenon. This intra-

nuclear mother organism, the product of conjugation, finally grows to a size of 10 to 12 microns and forms within it from eight to twenty "primary sporoblasts." The young sporoblasts are eventually liberated from the mother cell and are at first solid and homogeneous, like the gemmules, but later when they have reached a size of 1½ to 2 microns small vacuoles appear in the peripheral ring of substance and in each vacuole a young spore is formed. The formation of these spores terminates the "primary nuclear phase" of the organism. These spores, still within the nucleus of the epithelial cell, become, in their turn, sporoblasts, and the formation of a large number of secondary spores within them constitutes the secondary nuclear phase of the parasite. In the meantime the nucleus of the epithelial cell has degenerated, and the secondary sporoblast with its contained spores escapes first into the cytoplasm and eventually into the pericellular space. In accordance with this conception the intranuclear process is well calculated to give rise to a massive number of young parasites within the body. Councilman, Magrath and Brinckerhoff state that after the tenth day of the disease the parasites become more and more difficult of recognition by microscopic methods. However, Brinckerhoff found that even the desiccated crusts of pustules and vesicles produce typical lesions on the cornea of the rabbit. These forms have never been recognized positively in the blood of patients, and Magrath and Brinckerhoff were not able to produce lesions in the rabbit's cornea by inoculation of variolous blood. The general distribution of the lesions in the skin and the occurrence of fetal smallpox gives us abundant reason for believing that the blood stream is invaded by the parasites.

Cytoryctes in Vaccinia.

It was stated above that bodies of the general nature of those described are found in vaccinia as well as in smallpox, and this occurrence is some added reason for believing that *Cytoryctes variolæ, s. vaccinæ*, is the cause of these processes. It is a most interesting and important observation by the American authors cited that the intranuclear stage of the parasite does not occur in vaccinia (Tyzzer), and we are led to believe that this is an important differential point between

vaccinia and smallpox. Assuming that the bodies in question cause the disease, the thought is pertinent that the difference in virulence between vaccinia and variola inoculata may depend on the failure of the intranuclear cycle to appear in vaccinia.

The work of Guarnieri, and particularly that of Councilman, Magrath and Brinckerhoff, is most suggestive, and ardent supporters of their views have appeared with corroborative work (e. g., Howard). At the same time many skilled observers discredit entirely the parasitic nature of the bodies described, interpreting them rather as products of degeneration of the epithelial cells and nuclei or as inclusions of other tissue cells (e. g., leucocytes, Borrel) or fragments of other nuclei. Ewing expresses similar views. The state of the question is such that further study is urgently called for.

Infection Atrium.

We have no positive knowledge as to infection atrium in smallpox, although the existence of a "contagious zone" of atmosphere about the patients is good ground for the belief that invasion takes place through the respiratory passages. The disease which follows introduction of the virus into the skin is spoken of as *variola inoculata,* and is much less severe than smallpox. We are also ignorant to a large degree of the means of excretion or dissemmination of the virus. Osler states that the virus "exists in the secretions and excretions and in the exhalations from the lungs and skin." The dried epithelial cells which are continuously thrown off are no doubt a most important means of dissemination. Infection may be transmitted by means of clothing or other materials which have been in contact with patients, and the disease may be carried to others from the sickroom by a healthy person.

Dissemination.

Epidemiologic experience teaches that the virus is one of great resistance and tenacity.

Cyclic Nature of Symptoms.

The incubation period in variola falls within the extremes of eight to twenty days, most commonly from nine to fifteen days. The stage of invasion, or the primary fever, terminates the incubation period, and during this time the initial rash appears, accompanied by moderate hyperleucocytosis. On the third to the fourth days the remission sets in, the number of leucocytes in the blood decreases to normal or below normal, and cutaneous lesions make their appearance, and in the course of forty-eight hours show a vesicular nature. When the umbilicated vesicles are changed into pustules the temperature again rises (secondary fever) and hyperleucocytosis again develops. This much only of the clinical picture is mentioned to emphasize the cyclic nature of the phenomena; one may well suspect that the organism causing such a disease undergoes particular phases of development which in some way are related to the well-known clinical cycle.

Variations in Virulence.

Epidemics are sometimes of so mild a character that the patients are not bed-ridden and may be found in the pursuit of their occupations in spite of well-marked eruptions. Such occurrences can be referred only to a virus of low pathogenicity. Even mild epidemics, however, may be accompanied by severe and fatal cases. Cases of ambulatory smallpox are most important factors in spreading the disease.

Distribution of Virus in the Body.

We have nothing more than presumptive knowledge concerning the distribution of the virus in the body aside from its occurrence in the skin and mucous membranes. We may feel certain, how-

ever, that the infection is systemic. The lesions of the skin are of such a nature that they are generally regarded as of embolic character, which presupposes blood infection; and transmission of the disease through the placenta is decisive proof of a general distribution of the virus at some stage of the process. The failure to cause vaccinia in the cornea of the rabbit by inoculating the blood of patients (cited above) may indicate that the virus is present in the blood in small quantity or that circulating organisms are eventually destroyed. The intoxication of smallpox is manifestly general.

Secondary Infections. In few diseases does secondary infection play so important a rôle as in smallpox. When the cutaneous lesions have become pustular they usually contain pyogenic cocci, although they may be absent. It is somewhat strange that streptococci are more often encountered than staphylococci, in view of the normal presence of the latter in the epidermis. Fatal cases are almost without exception accompanied by general streptococcus infections, and Councilman believes these organisms are more important as a cause of death than the specific virus.

Prophylaxis. Successful prophylaxis involves universal vaccination, in addition to special measures which are demanded in the presence of the disease: isolation of the sick until desquamation is complete, antiseptic baths, and disinfection and fumigation as currently practiced.

Discovery of Vaccination. Interesting matters of history are the facts that protective inoculation against smallpox was practiced in fairly ancient times by rather primitive races, and that Lady Mary Wortley Montague

introduced this method into Europe in 1718. This was not the vaccination in vogue to-day, however, but rather the inoculation of virulent virus from the pustules of the diseased into the healthy. As mentioned in one of the earlier chapters, this procedure commonly produced a mild type of disease (variola inoculata) which rendered the individual immune to virulent smallpox.

Jenner.

Everyone knows that the vaccination of to-day, i. e., the substitution of the virus of cowpox for that of smallpox, was the discovery of Jenner (1798), and we need offer no comments concerning its efficacy nor repeat the well-earned epithets which have been applied to the rare species of disbelievers. Nothing is more certain than that smallpox has ceased to be a world pest only because of the continued Jennerization of the race.

The essential points established by Jenner are the following: 1. That the vaccine disease casually communicated to man has the power of rendering him insusceptible to smallpox. 2. That the specific cowpox alone, and not other eruptions affecting the cow which might be confounded with it, has this protective power. 3. That the cowpox may be communicated at will from the cow to man, by the hand of the surgeon, whenever the requisite opportunity exists. 4. That the cowpox, once engrafted on the human subject, may be continued from individual to individual by successive transmissions, conferring on each the same immunity against smallpox as was produced in the one first infected directly from the cow (cited from S. W. Abbott).

For at least half a century following Jenner's

Humanized Lymph. discovery humanized lymph was used for vaccination, new patients being inoculated by means of points prepared from vesicles of previous cases, or with the fresh lymph from such cases. The not infrequent transmission of syphilis by this means was the source of many calamities. Following the precedent of Warlemont in 1868, the lymph of cowpox is now the universal source of vaccine.

Cowpox. Cowpox probably occurs to a greater or less degree in all countries, especially in the spring and summer, attacks the udder and teats almost exclusively, and is accompanied by very mild constitutional symptoms. The incubation period is from three to eight days. There is first local heat, swelling and tenderness, followed by the formation of papules, which in three or four days after their appearance are transformed into vesicles. The disease reaches its maximum development at the tenth or eleventh day, the umbilicated vesicles going through the usual course to crust formation.

Preparation of Vaccine. Calves and heifers from the age of two months to two years are best suited for vaccination in the production of lymph for commercial purposes. The region of the flank or the whole ventral surface of the body may be inoculated, and in the latter instance as many as a hundred or more insertions may be made. The skin is first shaved, cleansed with antiseptics, and the lymph from another calf is introduced by means of a syringe or by scarification. In some institutions long, very superficial parallel incisions are made and the virus rubbed in with a spatula. Within five days to a week the vesicles are in such condition that the lymph may be collected, the contents either

being squeezed out with suitably formed forceps or scooped out with a sharp spoon. Depending on the area vaccinated, the lymph collected from a single calf may be sufficient for from 2,000 to 15,000 vaccinations in man. In view of the immunity which is conferred calves can be used but once for the production of vaccine virus.

Glycerinization.

All other methods of preserving lymph have been largely abandoned for the process of glycerinization, the glycerin being very intimately mixed with the virus by mechanical means and allowed to remain in this state in a cool place for from six to eight weeks before the product is put on the market. Dried vaccine on ivory points is still used to some extent, the points being coated directly from the vesicles. Dried vaccine retains its power for from two to four months or longer when kept in a cool, dark, dry place. Glycerinated lymph has many advantages, the most important of which relates to the bactericidal action of the glycerin by which the lymph is freed from the pathogenic bacteria (e. g., staphylococci) which in former times caused serious complications in vaccination. The glycerin is supposed to destroy such organisms to a large degree without, however, injuring the vaccine virus itself. It is also stated that glycerinated lymph is much more durable than the dried; that its potency may be retained for eight months or longer under suitable conditions. Rosenau has recently called attention to the fact that the bactericidal power of glycerin has been overestimated, and that while it kills pyogenic cocci within two weeks when at the body temperature, such organisms may live for months in glycerin when in the

Effect on Contaminating Bacteria.

ice chest; and, of course, our glycerinated virus is kept in the ice chest. Tetanus spores live for months in glycerin and glycerin has practically no neutralizing action on tetanus toxin. Glycerin does have the power, however, of attenuating the tetanus spores, and its slow bactericidal action is well established. As stated above, the vaccine should be glycerinized for some weeks before it is put on the market. Glycerin has the added advantage for the manufacturer of enabling him to dilute his lymph moderately (50 to 60 per cent.) without impairing the virus.

Of much more importance for the safety of virus than glycerinization are proper hygiene and cleanliness during the whole process of preparation. The powers recently conferred on the Surgeon-General by an act of Congress have resulted in a great improvement in the purity of the vaccine now on the market.*

While it can not be expected that vaccine will be entirely free from bacteria, it is possible to reduce their number to a low minimum and to eliminate pathogenic forms, particularly pathogenic cocci, tetanus and tubercle bacilli.

Vaccination

The technic of vaccination is so well known that no description is needed. It need only be stated that in scarifying it is undesirable to cause hemorrhage and that the operation is a surgical procedure, demanding surgical cleanliness and surgical care of the wound. As a rule vaccination in man protects against smallpox for a period of six to ten years, after which revaccination is necessary for continued protection. It

* John F. Anderson "Federal Control of Vaccine Virus," Jour. of the Amer. Med. Assn., June 10, 1905.

should not be concluded from the negative outcome of a single vaccination that the individual is immune to vaccination and hence immune to smallpox, but rather, repeated attempts should be made with virus known to be fresh. It is quite possible that certain individuals are immune to vaccinia, as often stated, but they are very rare, and the condition should not be recognized hastily. Among 38,000 vaccinations Dr. Cory encountered but one in which a "take" could not be gotten on second trial (Abbott).

When to Vaccinate.

The ideal condition is that all children should be vaccinated at an early age by requirement of law as in certain European countries, where it is demanded within the first few months or the first year or two of life. Some countries require revaccination before the children are admitted to school and recommend repetitions at suitable intervals.

We have no national law on the subject and the state laws differ. In many states children must be vaccinated before they are admitted to the public schools, the responsibility sometimes falling on the school and sometimes on the city or town authorities. A number of states have no laws on the subject, although vaccination is for the most part assured through the requirements of the State Boards of Health and the local authorities.

When there is danger of an epidemic, and in known cases of exposure, vaccination should be practiced thoroughly. Inasmuch as the incubation period of vaccinia is about three days less than that of smallpox, successful vaccination protects within a limited period following exposure. Immediate vaccination is demanded in case of

exposure. Healthy infants may be vaccinated within the first six weeks or two months of life and at any earlier period in case of exposure.

Immunity and Susceptibility. An attack of smallpox confers prolonged and, with few exceptions, lasting immunity. Second and even third attacks have been described. It is known that those who have had smallpox may become susceptible to vaccination after a period of time. Susceptibility varies a good deal with age. During the ages of from two to fourteen years the disease is less common than between fifteen and forty, and after this period it again decreases in frequency. Undoubted instances of natural immunity to smallpox occur, but they are very rare.

Leucocytes. Smallpox is accompanied by a leucocytosis which is peculiar because of the large number of mononuclears. There is a slight rise in the number of leucocytes during the first febrile onset, a fall to almost normal during the remission, followed by a second rise, which may be as high as 16,000 to 20,000. Fatal cases show a terminal hypoleucocytosis (Magrath, Brinckerhoff and Bancroft). Large numbers of lymphocytes are also found in the pustules (Roger). Nothing of a satisfactory nature is known concerning the relationship of the leucocytes to recovery and immunity.

Serum. There is no serum therapy for smallpox. The interesting observation has been made, however, that the serum of convalescents or of vaccinated man or animal will, when mixed with vaccine virus, prevent its action.

Horsepox is identical with cowpox. Sheeppox (clavelée) is an independent disease. The virus of cowpox produces a local lesion in the sheep, but does not

cause immunity to sheeppox. The virus of sheeppox, on the other hand, has no effect on horses and cattle (Nocard and Leclainche). The virus of sheeppox is filterable (Borrel).

VIII. CHICKENPOX (VARICELLA).

Although the skin manifestations of varicella often resemble those of smallpox to such an extent that differentiation is difficult, the two diseases are distinct. Nothing indicates this more clearly than the fact that one who has recovered from varicella is susceptible to vaccination, and it is known further that an attack of chickenpox does not protect against smallpox.

The etiology is unknown, and no organism which has been described can be considered the probable cause.

Varicella occurs epidemically and sporadically. The virus probably exists in the lesions of the skin and in the scales, and the latter may be the chief source of contagion. There is no definite knowledge concerning the resistance of the virus, nor its distribution; the conclusion is justified, however, that it exists in the circulation at least in an early stage of the disease. The infection atrium likewise is a matter of conjecture, but probably is to be found in the lungs or upper respiratory tract.

The patients should be isolated and school children should not be allowed to return to school until desquamation is complete. Disinfection should be practiced.

Susceptibility and virulence would seem to vary, since the severity of the cutaneous lesions is not constant. In delicate and tuberculous children, the lesions may become gangrenous. Hemorrhagic

varicella is observed occasionally. Such complications as nephritis and otitis media occur.

Varicella is a disease of childhood, although it may occur in adults. Infants are attacked less frequently. Second or even third attacks occur, although they are rare.

There is no serum therapy.

IX. SCARLET FEVER.

The rôle which the streptococcus plays in scarlet fever was considered on page 361.

The "bodies" recently observed by Mallory may be referred to briefly.

The Protozoon(?) of Mallory.

In 1903 Mallory described certain protozoon-like bodies found in the skin of four cases. They could be divided into two groups, one of which consisted of "round, oval, elongated, lobulated bodies" from 2 to 7 microns in diameter; the individuals of the second group "contain a central round body, around which are grouped, on optical section, from 10 to 18 narrow segments, which, in some cases, are united, but in others are sharply separated laterally from each other." They occur within and between the epithelial cells and in the superficial part of the corium. He gives the name of *Cyclaster scarlatinalis* to these bodies, and, although expressing the belief that they are protozoa and have a causal relation to scarlet fever, does not claim to have proved such a relation.

Duval corroborated the discovery of Mallory, and demonstrated the bodies in five out of eighteen cases in blisters which were produced artificially during the height of the eruption. Field found them not only in the skin of scarlet fever, but also in that of measles and concludes that many of

them at least represent artifacts or degeneration forms of tissue cells. More extensive observations seem to be necessary to establish the nature of these supposed parasites.

Other micro-organisms which have been described as the cause of scarlet fever, including the *Diplococcus scarlatinæ* of Class, we may pass over with the remark that the claims concerning them have not been upheld.

Contagiousness and Transmission.

The contagiousness of scarlet fever is extreme, and the virus undoubtedly is thrown into the surrounding air from the skin of the patient. It is highly probable that the virus also reaches the surrounding air from the respiratory passages by means of "drop infection," since transmission may occur before the skin shows involvement. Patients continue to be infectious for from 4 to 6 weeks or longer after the appearance of the eruption. The disease may be transmitted by an intermediate healthy person, or by contaminated clothing or furnishings. The origin of epidemics from milk which has in some way been contaminated seems to have been proved in a number of instances.

Of great importance for the persistence of an epidemic is the resistance of the virus, which remains viable and virulent for months and possibly for years, when under suitable conditions.

Prophylaxis.

Prophylaxis demands isolation of the patients until desquamation is complete; the use of antiseptic baths or ointments, or vigorous scrubbing with soap as desquamation proceeds; antiseptic treatment of the mouth cavity; disinfection of all utensils, linen, etc., with which the patient has been in contact; avoidance of stirring up the dust in the room, which demands moist rather than dry

cleansing; the disinfection of the sputum and other discharges of the patient; an abundance of fresh air and sunshine in the sick room; the final disinfection of the room. Physicians and nurses, when in the presence of the patient, should wear long gowns, which can be discarded on leaving, and other well-known precautions should be observed to avoid spreading of the disease.

Susceptibility and Immunity. Scarlet fever is particularly a disease of childhood, "a large proportion of cases occurring before the tenth year" (Osler). Adults are attacked not infrequently. Infants are less susceptible than older children. Many examples of family immunity, which probably is relative, are encountered, and likewise instances in which there is a family susceptibility. In a given family examples of individual immunity and susceptibility are frequently met with. One attack usually confers immunity against a second, but not invariably.

Leucocytes. Scarlatina is characterized by a leucocytosis, the degree of which bears some relation to the severity of the infection. In mild cases the average is from 10,000 to 18,850 (Bowie), in moderately severe cases from 20,000 to 40,000, or even as high as 78,000 (Klotz); in malignant uncomplicated cases there is a tendency to a low leucocytosis (Klotz). How much of this leucocytosis depends on co-existing streptococcus infection remains uncertain.

Serum Therapy. Treatment with antistreptococcus serum is the only serotherapeutic measure which has been advocated in relation to scarlet fever. This is done either on the assumption that the disease is of streptococcus etiology, satisfactory proof of which has not yet been obtained, or with the hope that

the serum will influence favorably secondary infections with the streptococcus. The serums of Aronson, Moser and of Menzer have been tried more than others. Moser is probably more enthusiastic than others, and he claims a reduction in the mortality from an average of 13.08 per cent. to 8.9 per cent. in 400 cases. Others have observed a favorable influence in some cases, but the results are not uniform. The development of secondary streptococcus infections can not be prevented by the use of the serums, although it is stated that their severity may be moderated.

Of theoretical interest is the report by Weissbecker and by v. Leyden that the serum of convalescents causes a reduction of the temperature and a shortening of the course of the disease.

The results published up to the present time indicate that we have not as yet an efficient serum for scarlet fever (see also p. 367).

X. MEASLES.

Micro-organisms.

Bacilli which have been recognized in the conjunctiva, sputum and nasal passages in cases of measles have, for the most part, resembled either the diphtheria or the influenza bacillus. Pseudo-diphtheria bacilli are normal residents in the eye, and influenza-like bacilli are found in the sputum in various conditions; hence, there is insufficient reason to associate such organisms with the etiology of measles. The micrococci found by Lasage (1900) have not received recognition as the cause of the disease.

Distribution of the Virus.

Measles is highly contagious, even during the prodromal stage. The contagion doubtless is excreted from the lungs as well as the skin, and, in

view of the early bronchial symptoms, the virus probably gains entrance through the lungs. Successful inoculation into man with blood taken from the involved skin shows that the virus exists in the circulation of the skin. Hektoen doubts the decisiveness of a number of these experiments since they were carried out in the presence of epidemics and natural infection could not be excluded; at the same time he does not question the results of Mayr (1852). In two experiments on man Hektoen determined the presence of the virus in the blood. "The results of these two experiments permit the conclusion that the virus of measles is present in the blood of patients with typical measles some time at least during the first 30 hours of the eruption; furthermore, that the virus retains its virulence for at least 24 hours when such blood is inoculated into ascites-broth and kept at 37° C. This demonstration shows that it is not difficult to obtain the virus of measles unmixed with other microbes and in such form that it may be studied by various methods." The virus is much less resistant than that of scarlet fever. The varying grades of severity of different epidemics show that it is subject to alteration in its virulence.

Effect on Resistance. Although measles is considered somewhat harmless on the whole, dangerous complications, such as broncho-pneumonia and otitis media, are sufficiently frequent. The development of tuberculosis following measles, an event which is not uncommon, shows that measles may greatly decrease general resistance.

Prophylaxis. The prophylaxis of measles is not different from that of other exanthemata. The isolation should continue for four weeks after the appearance of the

exanthem (Gotschlich). The sickroom should be disinfected eventually. The view not uncommonly encountered that measles is a good thing for a child to have and be over with is in no way justifiable. The development of serious complications can in no case be foreseen, and fatalities may occur even in mild epidemics.

Susceptibility and Recurrence

Very young children, the rachitic and tuberculous, and those in a poor state of nutrition should be guarded against exposure, for in them measles is often malignant. Infants are less susceptible than older children. Measles occurs in adults more frequently than scarlet fever. Recurrences, on the whole, are frequent, as many as four attacks having been noted in an individual. Hence, the immunity caused by infection is not uniformly of a permanent character.

Racial Immunization.

It is very probable that the inhabitants of a country in which measles is endemic gradually become immunized, with the result that the disease prevails in a mild form. On the contrary, regions in which measles has hitherto been unknown, or has been absent for many decades, are susceptible to visitations of great malignancy. Such epidemics have occurred in the Faroe Islands and in Iceland, with a mortality exceeded by few epidemic diseases.

Leucocytes.

A moderate leucocytosis is excited in measles, "which begins soon after infection, reaches its maximum six days before the appearance of the eruption, and lasts into the first part of the stage of invasion" (Tiliston). We are ignorant of the significance of this phagocytosis.

There is no serum therapy for measles. Weiss-

becker states that the serum of convalescents influences the course of the disease favorably.

XI. GERMAN MEASLES (RÖTHELN).

Rötheln is considered as distinct from measles, in spite of clinical similarities. It is recognized because of certain peculiarities in the eruption and its uniformly mild course. Perhaps the strongest reason for believing the two diseases to be distinct lies in the fact that an attack of rötheln does not leave an immunity against measles.

Rötheln is contagious. Efforts should be made to prevent extension, as in measles, the methods of transmission being the same in the two diseases.

XII. WHOOPING COUGH.

Various protozoa (?) and bacteria (cocci and bacilli) have been assigned as the cause of whooping cough. Many of the so-called protozoa found in the throat were undoubtedly tissue cells (leucocytes, ciliated epithelium). Among the cocci, the diplococcus of Ritter (1892) acquired some prominence. He is said to have found it constantly in 146 cases. Investigations by others failed to justify his conclusions.

The Influenza-like Bacillus of Sprengler and of Jochmann.

Disregarding some other bacilli which certain investigators have attempted to bring into relation with pertussis, we may note the essential facts concerning an influenza-like bacillus which has been found with great constancy and by many competent investigators in the sputum of patients. First observed by Sprengler (1897) in pertussis sputum, this organism or bacilli similar to it have been found by Czaplewski and Hensel, Zusch, Cavasse, Vincenzi, Elmassian, Luzzatto, Arnheim, Jochmann and Kruse, Reyher, Smit, Wollstein,

and Davis. The organism is said to be somewhat larger and thicker than the true influenza bacillus, but has the same bipolar staining affinity and the same demand for hemoglobin for its growth in pure cultures. There is some difference of opinion as to whether the organisms described by these different observers are all identical and as to whether all have worked with pure cultures. The conclusion of Davis would seem to sum up the situation: "With the exception of Manicatide, probably all of the investigators, at least in more recent years, have been dealing, either in pure or impure cultures, with the influenza-like bacillus, first described by Sprengler and later by Jochmann." Culturally they are not to be differentiated from the influenza bacillus. When in pure culture they demand hemoglobin for their development, although the amount of hemoglobin may be so small as not to color the medium. When in mixed culture with the streptococcus, staphylococcus, pneumococcus and *B. xerosis,* they grow abundantly even in the absence of hemoglobin. Hence, in relation to symbiosis, also they resemble the influenza bacillus. For symbiotic development it is necessary that the secondary organisms be living; when killed or when the filtrates of bouillon cultures are used, the "pertussis bacilli" are not stimulated to growth.

Hemophilic Properties and Symbiosis.

Inoculation of pure cultures on the mucous membrane of the upper respiratory passages in various animals, including the monkey, does not produce a pertussis-like infection. The organisms have, however, a low degree of virulence for animals, particularly the guinea-pig. Davis found that three blood-agar cultures injected intraperi-

Pathogenicity.

toneally killed guinea-pigs in 24 hours or less. The virulence of the organism is augmented when mixed with certain other bacteria. By injecting it, mixed with a non-pathogenic staphylococcus, its virulence, after six passages, was so increased that one blood-agar culture killed guinea-pigs in 24 hours (Davis). In this respect, also, it resembles the influenza bacillus.

Significance. Inoculated in the throat of an adult, who presumedly had never had whooping cough, a distinct febrile reaction, lasting two or three days, developed after an incubation period of two days (Davis). Headache and pharyngitis were accompaniments of the reaction and the pharyngitis continued for at least four weeks. There was little cough, and it was concluded that the microorganism had not produced whooping cough, although it had shown toxic and infectious properties. The bacillus proliferated enormously in the pharynx and nose and was still to be cultivated after four weeks. Such an organism may well be an important factor in whooping cough, even though it is not the essential cause. Davis is inclined to regard its relation to whooping cough as similar to that of the streptococcus to scarlet fever—i. e., a very important complicating organism.

Davis finds still further reason for doubting its specific relationship to whooping cough from the fact that it was found frequently in measles, acute influenza, epidemic meningitis, bronchitis, varicella and in normal throats.

The organism is disseminated extensively by coughing, and the same is probably true of the essential virus. Close contact, as by kissing, or the

common use of eating utensils is a means of transmission. The opinion has been advanced by Weill and Pehn that pertussis is contagious only during the catarrhal stage of the disease. "Of ninety-three non-immune children who were placed with fifteen children who were in the convulsive stage, none became sick" (cited by Gotschlich). This point is not sufficiently established, however, to warrant modifications of prophylactic measures. Whooping cough is often epidemic and is more common in cities where contact with the infected is more likely to occur than in the country. The incubation period is from seven to fourteen days. **Contagiousness.**

Isolation is more difficult than in the more acute contagious diseases, yet contact with other children should be avoided as much as possible, and the patients should be withdrawn from school until recovery is complete.

Pertussis is almost exclusively a disease of children, although older people may be attacked. Susceptibility is not general. One attack usually confers immunity. A varying degree of leucocytosis is excited by the infection (12,000 to 45,000), the significance of which is not known. It is chiefly mononuclear.

Serum therapy for whooping cough has not advanced to a point where we can speak with assurance concerning it. Manicatide (1903) immunized horses and sheep with the organism which he cultivated from a large number of cases. He reports that cure may be accomplished in from two to twelve days when the serum is used within the first fifteen days of the disease. The bacillus of Manicatide differs from the influenza-like organism of other observers, hence, his antiserum can **Serum Therapy.**

not be accepted unreservedly as a specific serum for whooping cough. Smit found that an antiserum for the influenza-like organism exerted no influence on the disease.

XIII. MUMPS (EPIDEMIC PAROTITIS).

Mumps occurs epidemically in children, particularly in schools, in other institutions, and in soldiers confined to barracks. It is most frequent in the spring and autumn and probably is endemic in large centers of population. It is contagious, the virus probably being disseminated from the upper respiratory passages with infected droplets of sputum and saliva. The disease has an incubation period of two to three weeks and runs its course in from seven to ten days.

Involvement of the testis, ovary or female breast are complications to be feared in adult life; "orchitis, albuminuria, with convulsions, acute uremia, endocarditis and peripheral neuritis are occasional complications" (Osler). Fatal meningitis develops rarely. Very young infants and adults are attacked less frequently than children of school age.

Patients should be isolated for three weeks from the time symptoms appear (Gotschlich).

One attack usually establishes protection.

A peculiar form of parotitis sometimes follows injury of the abdominal and pelvic viscera, the so-called "postoperative" parotitis. The parotid glands may also be invaded by a number of known micro-organisms, e. g., the pneumococcus.

APPENDIX.

I.—The Hypothesis of Welch.

What has come to be known as the hypothesis of Welch is of such practical and theoretical importance that reference to it should not be passed over. It may be put in the form of the following question: If bacterial toxins and the constituents of bacterial cells so act on the tissue cells that the latter produce bodies (antibodies) which are inimical to the bacteria, why may not the body fluids in turn so act on the bacteria that the latter produce bodies (antibodies) which are inimical to the tissue cells? "Looked at from the point of view of the bacterium, as well as from that of the animal host, according to the hypothesis advanced, the struggle between the bacteria and the body cells in infections may be conceived as an immunizing contest in which each participant is stimulated by its opponent to the production of cytotoxins hostile to the other, and thereby endeavors to make itself immune against its antagonist." (Welch.)

A more reasonable hypothesis could hardly be advanced, and no small number of facts known at the present time are in harmony with it. Walker had already performed work of a fundamental character, which showed that the typhoid bacillus, when grown in the presence of its antiserum, acquires greater virulence for animals. Furthermore, a greater dose of protective serum was required to save guinea-pigs from infection with the immunized culture than from the same strain which had not been immunized. The fact has been known for a long time that the typhoid bacillus resists agglutination when freshly cultivated from a patient having the disease, whereas it becomes easily agglutinable after a period of artificial cultivation. It may well be assumed that the bacillus, when playing the part of an infecting organism, gradually was immunized against the agglutinating properties of the patient's serum; and, on the

other hand, that it lost this resistance after it had been removed from the stimulating influence of the infected body. This immunization with agglutinins may be carried on in the test glass, and bacteria which have been so treated acquire the power to absorb a greater quantity of agglutinin from the homologous serum (Bail).

Another pertinent observation was that by Wechsberg, who found that a strain of the diphtheria bacillus when grown in a medium containing diphtheria antitoxin could be made to produce diphtheria toxin more abundantly. We may assume that the antitoxin combined with the corresponding receptors situated in the bacilli (diphtheria toxin), and that the bacilli were, as a result, stimulated to produce a greater number of such receptors (toxin).

Consistent as these observations are with the hypothesis under discussion, Welch meant a great deal more than the immunization of the bacteria against the defensive powers of the animal body. Not only may a bacterium during infection become more resistant to the bactericidal action of the body by producing antibodies for those bactericidal agencies, or by its ability to absorb and dispose of a greater quantity of bacteriolysin; and not only may a bacterium be able to respond to the presence of natural antitoxins in the body by the production of more toxin; but, in addition, certain constituents of our body fluids may, by combining with suitable bacterial receptors, stimulate the bacterium to the production of a whole shower of cytotoxins, which attack the leucocytes, erythrocytes, nerve cells, liver, kidney, etc. The nature of the animal substances which may combine with the bacterial receptors and thus cause the formation of the bacteriogenic cytotoxins is left an open question, and is not of essential importance for the theory; it is not at all necessary that they be toxic for the bacterium, and they may even be taken up as food substances. Likewise the possible nature of the cytotoxins produced by the bacterium is of secondary importance. It so happened that Welch assumed that they might be of the nature of amboceptors, which may become complemented by bacterial complement, by the circulating complement of the body or by endocomplements of the tissue cells. One could with equal reasonableness assume that

they may be complete toxins, receptors of the second order, with a haptophorous and a toxophorous structure.

A well-known statement of Metchnikoff is to the effect that a particular bacterium when virulent is not so readily taken up by leucocytes as is an avirulent strain. This fact has been noted repeatedly in recent times in the study of phagocytosis in the test tube. This may be because the organism, in its virulent parasitic state, secretes substances which repel the phagocytes, neutralize the opsonins, or because of the formation of actual leucocytic toxins.

One of the most widely known phenomena in relation to the virulence of some organisms is that their pathogenicity may be increased by passing them through suitable animals repeatedly. The best results are obtained when intermediate artificial cultivation is avoided and the inoculations are made directly from the dead into the living animal. It may, with all reason, be assumed that by continued residence in the host the bacterium has been trained to produce a greater quantity of toxic substances which are inimical to the host, and that the increased virulence of the parasite depends on this condition.

Although up to the present time systematic attempts to place the hypothesis of Welch on a firm experimental basis appear not to have been made, the observations cited, as well as others which could be enumerated, provide cumulative evidence of its correctness.

II.—The Aggressins of Bail.

Not entirely foreign to the subject discussed above is the so-called aggressin theory of Bail, the essential points of which may be given without entering into a detailed discussion.

Bail attributes to pathogenic bacteria the property of "aggressiveness," through which they directly antagonize the protective agencies of the body. The micro-organisms of highest parasitic powers, the "true parasites," as those belonging to the hemorrhagic septicemia group, possess the greatest aggressiveness, since they are able to proliferate in the blood stream while the antibacterial activities of the body (phagocytosis, etc.) are held in abeyance. Other bacteria, which in causing disease tend

to remain localized, and, if by any means they reach the blood stream, are not able to proliferate greatly in this place, are "half parasites" and have a lower degree of aggressiveness; they are more susceptible to phagocytosis and to the action of bacteriolysins (typhoid, cholera, dysentery). Saprophytes have no aggressive action.

This is very general, but Bail and his co-workers have attempted to put the conception on an experimental basis by demonstrating the existence of a substance on which the aggressiveness of bacteria depends; to this substance they give the name of "aggressin."

Intraperitoneal inoculation of the tubercle bacillus into the guinea-pig leads to more or less general tuberculosis and to the death of the animal in the course of a few weeks. If, during the course of the disease, a second injection of a large quantity of the bacillus is made into the peritoneal cavity, or if an injection of tuberculin is given, the animal dies very quickly. This is, of course, nothing more than the well-known hypersusceptibility of tuberculous animals to the products of the tubercle bacillus. In addition to this fact, however, a similar result was obtained in another manner. If a large quantity of bacilli is placed in the peritoneal cavity of a healthy guinea-pig, and the exudate is removed after twenty-four hours and freed from leucocytes and bacilli, the aggressin of the bacillus is said to be present in the clear fluid. This is demonstrated by injecting some of the fluid, together with tubercle bacilli, into the peritoneal cavity of another healthy guinea-pig. The rapid death of the animal is the result, whereas the bacilli alone cause death only after a long period, and the cell-free exudate alone is without toxicity.

A similar condition has been found in experimental infections with a number of bacteria (typhoid, cholera, dysentery, plague, chicken cholera), the essential fact being the same: that, following intraperitoneal or intrapleural inoculation, the resulting exudate, when freed from leucocytes and bacteria, has the power of intensifying an infection by the corresponding organism.

There seems at present to be no definite knowledge concerning the nature of these aggressins, although Bail thinks they may resemble true toxins in some respects. Likewise the precise character of their action is un-

known, although Bail and his co-workers are strongly inclined to the view that they inhibit phagocytosis by some direct action of the leucocytes.

It is further interesting that immunization with aggressins is said to give rise to the formation of antiaggressins, and that by the use of antiaggressive serum the action of the aggressins is neutralized, and the bacteria consequently become the prey of the leucocytes. The action of the antiaggressive serum is said not to depend on the presence of bacteriolysins.

One can hardly attempt a serious criticism of the aggressin theory at this time, and the above statements are made only to signify its general character.

III.—Cobra-lecithid.

Too late to be incorporated in its proper place in the text, it is learned that the chemical identity and molecular weight of cobra-lecithid have been determined by Dr. Kyes in the laboratory of Ehrlich, and that work descriptive of the characteristics of the substance is in process of publication.

BIBLIOGRAPHY.*

Hopf: Immunität und Immunisierung, Tubingen, 1902.

Ehrlich (and his pupils): Collected Studies on Immunity. Authorized Translation by Charles Bolduan, M. D. (In preparation). John Wiley & Sons, New York.

Metchnikoff: Immunity in Infective Diseases, Masson et Cie., Paris, 1901. Translated by Binnie. MacMillan & Co. $5.25 net.

Dieudonné: Immunität, Schutzimpfung und Serumtherapy. 4th Edition. Joh. Ambr. Barth, Leipzig, 1905. (Gives the fundamental literature.)

Roger: Les Maladies Infectieuses. Masson et Cie., Paris, 1902. (Detailed treatise on the infectious properties of different micro-organisms.)

Kolle and Wassermann: Handbuch der Pathogenen Mikro-organismen, Gustav Fischer, Jena, 1902-1904. Four volumes.

Ritchie: Current Theories of Immunity. Journal of Hygiene, 1902, pp. 215, 252, 344.

Charrin: Les Défenses Naturelles de l'Organisms. Masson et Cie., Paris, 1898.

Bosanquet: Serums, Vaccines and Toxins. Keener & Co., Chicago, 1904. 344 pages.

Oppenheimer, Carl: Toxine und Antitoxine, Fischer, Jena, 1904. (Gives the important literature.)

Wassermann: Immune Sera, Hemolysins, Cytotoxins and Precipitins. Translated by Charles Bolduan, 1904.

Sachs: Die Hemolysine. From Lubarsch-Ostertag's Ergebnisse. Bergmann, Wiesbaden, 1902. (Literature to date of publication is given. Reprint may be purchased.)

Sachs: Die Cytotoxine des Blutserums. Biochemisches Centralblatt, i, 1903, 573 ff. (Literature to date of publication.)

Bensaude: Le Phénomène de l'Agglutination des Microbes. Paris, Carré et C. Naud, 1897. (Gives the early literature on agglutination.)

v. Dungern: Die Antikörper. Fischer, Jena, 1903.

Weigert, E.: Les Tuberculines. Storck et Cie., Lyon, France, 1902.

Wright: A Short Treatise on Antityphoid Inoculation. Archibald Constable, Westminster, 1904. (An exposition of the methods and principles of Wright.)

Scheube: Diseases of Warm Countries. Second Edition, 1903. Translated by Cantlie.

Manson: Lectures on Tropical Diseases. Chicago. Keener & Co., 1905.

Nocard and Leclainche: Les Maladies Microbienne des Animaux. Two volumes, third Edition. Masson et Cie., Paris, 1905.

Welch: The Huxley Lecture on Recent Studies of Immunity with Special Reference to Their Bearing on Pathology. Medical News, 1902, vol. lxxxi, 721.

* See note in the Preface concerning Bibliography.

INDEX.

PAGE.

Abrin 6, 104, 204

Achalme, bacillus of, in rheumatic fever 359

Acne, staphylococcus in 376

Acquired immunity (see Immunity, acquired).

Active immunity (see Immunity, active).

Actinomyces bovis et hominis (ray fungus).....10, 458
Classification of, 459, 460; cultivation and morphology of, 459; lungs, in, 337; occurrence of, in nature, 460; phagocystosis of, 461; resistance of, 459; species of, and virulence of, 460.

Actinomycosis 10, 450-462
Animals, susceptibility of, to, 460; connective tissue, formation of, in, 5, 458, 461; immunity and susceptibility to, 461, 462; infection atria, 460; iodid of potassium in treatment of, 462; lesions, character of, 458, 460; phagocytosis in, 461; prophylaxis of, 461; transmission of, 460.

Acute articular rheumatism (see Rheumatic fever).... 541

Adrenal gland, cytotoxin for..... 173

Agglutination 92, 118
Of erythrocytes, 103; of erythrocytes with silicic acid, 129; etiology, determined by, 7; group agglutination, 110, 113; immunity, relation to, 96, 97, macroscopic and microscopic, 102; prognostic importance of, 95; sodium chlorid, influence on, 110; stages in the reaction, 110; substances concerned, 105; serum dilutions, 113; specificity, 111; technic, 98; theories of mechanism of, 116; see also under Agglutinins and under different diseases and micro-organisms.

Agglutinins 92-118
Absorption of by bacteria, 203; agglutinophorous group, 108; autoagglutinins, 104; chief agglutinins, 111; congenital, 93, 96; definition, 105; distribution of, in the body, 95; Ehrlich's theory of the production of, 114; ferments, action of, on, 96, 107; formation of, following vaccination, 233; haptophorous group of, 108; *Hauptagglutinin,* 111; immune, 62, 93; isoagglutinins, 104; mixed infections, influence of, on, 113; *Mitagglutinin,* 109; normal, 55, 92; origin of, 96; precipitation of, by chemicals, 107; production of, 93; receptors of 2d order, 108; resistance to acids and alkalies, 109; resistance to heat, 97, 108, 109; somatic and flagellar, 107; specificity of, 92; structure of, 108; union with cells, character of, 203, 204; unit of measure of, 103; variations of, in animals, 114; variations in the quantity of, 95; zymotoxic group of, 108; see Agglutination, Agglutinogens, Agglutinoids, and also under the different micro-organisms.

PAGE.
Agglutinogenic power of bacteria..... 94
Agglutinogens, or agglutinable substances 105
Diffusibility of, 107; distribution of, 106; flagellar and somatic, 107; multiplicity of, 107; resistance of, to heat, 107; structure of, 108; see Agglutinins and Agglutination.
Agglutinoids 109
Aggressins 569
Alexins 49, 130
Definition of, 49; identity of, with complement, 134, 135; nature and selective action of, 131; see Complement.
Alkaloids.
Failure to cause formation of antibodies, 198; state of, within the cells, 198, 205.
Amboceptoid, ... 209
Amboceptors 134, 141
Absorption of, by cells, 144, 146, 203; bacteriolytic, 143; complementophilous haptophore of, 147; cytophilous haptophore of, 146; formation of, 150; formation following vaccination, 233; hemolytic, 141; influence in phagocytosis, 190; isolation of, 146; manner of action of, with complement, 145, 148, 207, 208; occurrence of, in animal secretions, 158; origin from leucocytes, 183, 190; origin in cholera, 190; receptors of the 3d order, 207; sensitization by, 142, 145; solutions of, 144; specificity of, 152, 153; structure of, 147; synonyms for, 148, 217; union with cells, nature of, 147, 148, 203, 204; see Hemolysins (serum), Bacteriolysins, Cytotoxins and Venoms.
Ameba coli.
Discovery of, 502; pathogenicity of, 502; symbiosis of, 502; see Amebic dysentery.
Ameba proteus 500
Ameba.
Cultivation and distribution of, 501; phagocytic action of, 176; resistance of, 501; symbiosis, 501, 502.
Amebic dysentery 500
Anatomic changes in, 503; immunity to, 504; liver abscess in, 503; occurrence of, 502; prophylaxis, 503; see Amebæ, and *Ameba coli.*
Amibodiastase 176
Amylold degeneration, production of by staphylococcus.. 375
"Anatomic tubercle" (see Tuberculosis).
Animals, susceptibility of, to
Actinomycosis, 460; anthrax, 330, 331; *B. influenzæ,* 394; *B. melitensis,* 335; cholera, 309; hydrophobia, 513, 514; leprosy, 447; *Micrococcus catarrhalis,* 384; *Micrococcus meningitidis,* 390; oidiomycosis, 467; pneumococcus, 339; pseudotuberculosis, 444; relapsing fever, 404; staphylococcus, 375, 376; streptococcus, 352; syphilis, 522; trypanosomiasis, 490, 495; tuberculin, 414; tuberculosis, 427, 441.
Animal experiments, in testing value of serums..... 201
Anopheles mosquitoes.
A. maculipennis, 478; *A. punctipennis,* 478; habits of, 478; life cycle of, 478, 479; malaria, rôle in, 468; migration of, 479; occurrence, 478.

PAGE.
Anthracase-Immunproteidin 332
Anthrax 327-333
Animals, immunity and susceptibility of, 330, 331; bacillemia, 330; discovery of its microbic nature, 27, 28, 29; immunity, 332; immunization, mixed, 333; influence of streptococcus on, 362; malignant pustule, 329; occurrence, 327; opsonins, 331, 333; phagocytosis in, 190, 331; prophylaxis, 330; serum-therapy, 332; toxic results, 330; transmission, 329; vaccination, 28, 29, 332; wool-sorters' disease, 329; see also *B. anthracis.*
Antiabrin 90
Antiaggressins 571
Antiamboceptors 156
Danger in formation of, 157; as receptors of the first order, 207.
Antibacterial serums (see Bacteriolysins) 226-231
Antibodies.
Mechanism of production, 199; origin of, 210, 314; scheme of, 216; specificity of, 208; union with antigens, 200; see Antitoxins, Amboceptors, Agglutinins, Precipitins, Hemolysins, Bacteriolysins and Cytotoxins.
Anticomplements 154, 207
Anticrotin 90
Anticytotoxins 165
Antiferments 63, 90
Antigens.
Scheme of, 216; union with tissue cells, character of, 200.
Antiglobulin 124
Anti-immune serum 156
Antilaccase 90
Antileucocidin 90, 372
Antileucotoxic serum 169
Antinephrotoxin 170
Antineurotoxin 171
For venom, 266.
Antipepsin 90
Antiprecipitins 123
Antirennet 90
Antiricin 90, 201, 202
Antirobin 90
Antispermotoxin 166
Antistaphylolysin 380
Antisteapsin 90
Antistreptocolysin 353
Antitoxins 65, 91, 221, 235
Early administration of, 223, 224; curative action of, 222, 223; discovery of, 33; examination of by U. S. Hygienic Laboratory, 76; for animal toxins, 90; for *B. botulinus,* 359; for *B. diphtheriæ,* 241, 243; for *B. pyocyaneus,* 260, 261; for *B. tetani,* 223, 252; for bacterial toxins, 89, 90; for plant toxins (abrin, crotin, ricin, robin, phallin), 90, 264; for pollen toxin, 263; for zoötoxins, 268; formation of, 85; haptophorous group of, 79; infections characterized by the formation of, 235, 268; leucocytic

PAGE.

origin, question of, 191; manufacture of, 69; mode of action of, 201, 221, 226; nature of, 191; toxins, neutralization of, by, 78, 201, 203; normal, 45; preservation of, 71, 73; prophylactic action of, 226; receptors, free, 89; receptors of the first order, 207; relation of, to toxins, in the body, 222; relation of, to toxins, in vitro, 221; standardization of, 72, 255; unit of, 72; see Part II, Group 1, and also the different micro-organisms.

Antitrypsin 90
Antiurease 90
Antivenin 70, 90, 267, 268
Antityrosinase 90
Arachnolysin (spider poison) 16, 268
Arrhenius and Madsen, views of 210
Arthritis 344, 348, 354, 378, 386, 391
Aspergillus 6, 467
Atrophy, phagocytosis in 178, 179
Attenuation.
Importance of in vaccination, 57; methods of, 219.
Autoagglutinins 104
Autocytotoxins 163, 174
Autolytic products, vaccination with 220, 312
Autonephrotoxins 169
Autoprecipitins 121
Autospermotoxin 166
Bacillus aërogenes capsulatus 14, 185, 359
Bacillus alcaligenes 270
Bacillus anthracis 10, 327-329
Antagonism of, by other bacteria, 329; antiserums for, 32; attenuation of, 58, 219; cultivation of, 328; discovery of, 27, 327; gastric juice, effect of, on, 39, 329; immunity, active, 332; immunity, acquired, 186, 331; immunity, natural, 330; immunity, passive, 332; infection atria, 39; opsonins, 331; phagocytosis of, 331; serums, effect of, on, 48, 49, 331; spores of, 29, 327, 328; toxic properties of, 330; virulence of, 329; see Anthrax.
Bacillus botulinus 256, 257
Animals, susceptibility of, 257, 258; antitoxin for, 259; morphology, etc., 257; occurrence in meat, 257; saprophytic nature of, 258; spores of, 257; toxin, action of, 258; toxin, detection of in meat, 257; toxin, preparation and resistance of, 258; see Botulism.
Bacillus chancri mollis (bacillus of Ducrey; bacillus of soft chancre) 16, 399
Cultivation, morphology, phagocytosis of, susceptibility of animals to, 400.
Bacillus of chicken cholera 58
Bacillus coli communis 298-304
Agglutination of, 92, 94, 111, 304; antagonism for putrefactive bacteria, 299, 300; antiserums, properties of, 303; beneficial functions of, 299; in cystitis, 303; in enteritis, 40, 301, 303; group agglutination, 111; group of, 298; in meningitis, 389; morphology and staining of, 298; occurrence in intestines, 298, 299; occurrence in nature, 298; in pneumonia, 336; re-

PAGE.
sistance of, 298, 299; serums, effect of, on, 299; symbiosis with *Ameba coli*, 502; toxin of, 303; typical strains of, 299; virulence of, 300, 301, 302.
Bacillus diphtheriæ 10, 235, 236
Agglutination of, 243; antitoxin for, 89, 241, 242; morphology, staining, cultivation, resistance, viability of, 236; occurrence of, in the body, 237, 238; phagocytosis of, 240; pneumonia, in, 336; toxic action of, 15; toxins of, 66, 67, 237, 238, 242; toxin, attenuation of, 40, 219; tuberculosis, in, 425; see Diphtheria.
Bacillus of Ducrey. See *Bacillus chancri mollis*.
Bacillus dysenteriæ 10, 288-290
Agglutination of, 92, 94, 288, 289, 294; antiserums for, properties of, 293; cultivation and morphology of, 288, 289; dissemination of, 292; endotoxin of, 291; etiologic rôle of, 289; "Flexner" type of, 289; pseudodysentery bacilli, 288; toxicity of, 291; toxin, autolytic, of, 291; types of, 288; see Dysentery, acute epidemic.
Bacillus edemæ malignæ 1, 14
Bacillus enteritidis 294-298
Agglutinins and agglutination of, 94, 298; *Bacillus paratyphosus*, resemblance to, 285; discovery of, 295; fermenting powers of, 295; group agglutination, 111; group of, 295; meat poisoning by, 294-298; morphology and staining of, 295; occurrence of, in meat of horses and cattle, 295, 296, 297; poisoning by oysters and fish, in, 297; resistance of, 297; toxin, 295-296; toxin, occurrence in meat, 297; toxin, resistance of, 297.
Bacillus of Friedlander; see *Bacillus pneumoniæ*.
Bacilli from butter, grass and milk 444
Bacillus icteroides, in yellow fever..... 11, 530, 531
Bacillus influenzæ 10, 394
Agglutination of, 399; animals, virulence for, 395; antiserum, properties of, 399; in conjunctivitis, 396; cultivation of, 394; discovery of, 394; excretion of, 395; hemophilic properties of, 394; immunization with, 399; in meningitis, 389-396; morphology and staining of, 394; occurrence of, in the body, 396; otitis media, in, 396; peritonitis, in, 396; resistance of, 395; symbiosis of, 394; toxin of, 395; tuberculosis, in, 425; see Influenza.
Bacillus lactis aërogenes.
Antagonistic action on putrefactive bacteria, 300; occurrence in intestines, 401.
Bacillus lepræ 10, 446
Animals, insusceptibility of, to, 447; antiserums for, 452; discovery of, 446; endotoxin, question of, 450; excretion and occurrence in nature of, 447; incultivability of, 447; morphology of, 447; occurrence in the body, 449; phagocytosis of, 449, 451; see Leprosy.
Bacillus of Lustgarten 443, 552
Bacillus mallei 10, 453
Agglutination of, 458; cultivation, morphology and resistance of, 453; mallein, varieties, and prepara-

PAGE.
tion of, 454; meningitis, in, 389; phagocytosis of, 456.
Bacillus melitensis 334, 335
Agglutination of, 334; animals, susceptibility of, to, 335; morphology of, 334; opsonins, influence of in phagocytosis of, 334; serums, effect of, on, 334; see Malta fever.
Bacillus mucosus capsulatus 401
Bacillus of ozena 402
Bacillus paratyphosus 284
Agglutination of, 11, 284, 287; antiserums for, properties of, 287; blood cultures, 288; endotoxin, 287; excretion of, 286; meat poisoning by, 285; occurrence in the body, 286; "paracolon" bacilli, relation to, 285; resistance of, 286; toxicity, 287; types of, 285; see Paratyphoid fever.
Bacillus pestis 10, 316-319
Agglutination of, 94, 326; cultivation of, 316, 317; endotoxin, resistance of, 319; excretion of, 321; involution forms, 317; meningitis, in, 389; morphology, 316; phagocytosis of, 325; pleomorphism, 316; pneumonia, in, 336; resistance and viability, 317, 318; staining of, 316; toxicity of cell bodies, 319; toxin of Lustig and Galeotti, 318; toxin, soluble, question of, 318; virulence, 318, 319; see Plague.
Bacillus pneumoniæ (bacillus of Friedlander). 337, 401, 402
Agglutination of, 94, 402; antagonism for *B. anthracis*, 329; antiserum, 402; influenza, 397; lesions caused by, 402; meningitis, in, 389; pneumonia, in, 336, 344, 402; tuberculosis, in, 425.
Bacillus prodigiosus.
Antagonism for *B. anthracis*, 329; Coley's mixture, in, 362; symbiotic action of, 185.
Bacillus psittacosis, agglutination of 94, 111
Bacillus pseudotuberculosis, varieties of 445
Bacillus pyocyaneus 259-261
Agglutination of, 92, 94; agglutinins for, 261; agonal invasion by, 259; antagonism for *B. anthracis*, 329; antitoxin, 261; bactericidal serum for, 261; ferments of, 260; endocarditis, in, 259; endotoxin of, 260; enteritis, in, 40; infections, symptoms of, 260; meningitis, in, 259; pigments of, 260; pyocyanase, 260; pyocyanolysin, 260; pyocyanin, 360; secondary infections by, 259; septicemia, in, 259; toxic action of, 16; toxin, soluble, 66, 260, 261; tuberculois, in, 425.
Bacillus of rhinoscleroma 402
Bacillus of symptomatic anthrax 185
Bacillus tetani 244-256
Agglutination, 256; anaërobic property of, 247; animals, susceptibility of, to toxin, 51; avirulent strains, 249; discovery of, 244; morphology, staining, cultivation, 244, 245; occurrence in intestines, 246; occurrence in nature, 245; parasitic power of, 247; pathogenic properties of, 249; resistance of spores of, 246; toxins of, 15, 20, 66, 67, 249; toxin, absorption of, by leucocytes, 191; toxin, fixation of,

PAGE

by tissues, 52, 53, 204, 205, 222, 251; toxin, attenuation of, 219; toxin, action of gastric juice on, 39; toxin, neutralization of, by antitoxin, 224; action of pancreatic juice on, 40; virulence, 185; see Tetanus.

Bacillus tuberculosis 10, 407

Agglutination, 438, 440; agglutination, relation of to immunity, 97; animals, susceptibility of to, 427; antiserums, properties of, 438; attenuation of, 410; avian, 442; bacteria resembling, 443; bovine, differentiation of bovine, from human, 417; constituents, 411; cultivation, 409; discovery of, 407; effect on tissues, 42, 44, 422-425; excretion of, 414, 415, 419; fever producing substance of, 411; of fish, 443; gastric juice, resistance to, 39, 410; immunization with, 411, 430-432; inflammation of lungs, in, 337; lesions produced by, 411; morphology of, 408; occurrence in nature, 414; pathogenic properties of, 411; phagocytosis of, 421, 422; proteins in, 411; resistance of, 409; staining properties of, 408, 411; streptococcus, influence of, on cultures, 356; "toxalbumin" of, 411; toxic substances, effects of, 411; toxin of Marmorek, 404; toxins, 439, 440; virulence of, 410, 427; see Tuberculosis.

Bacillus typhosus 10, 269

Agglutination of, 92, 94, 116, 283, 284; antitoxin, question of, 271; autolysis of, 271; blood cultures of, 273, 284; discovery of, 269; dissemination of, 270; endotoxin, 271; excretion of, 273, 274; extracts of, 282; gastric juice, action of, on, 39; immunization with, 280, 281, 283; leucocytes, relation of, to, 277; meningitis, in, 389; morphology of, 269; occurrence in body, 9, 270, 274; occurrence in nature, 270; phagocytosis of, 274; pneumonia, in, 336; resistance of, 270; symbiosis with *Ameba coli*, 502; toxin of Chantemesse, 282; vaccines, 279, 282; see Typhoid fever.

Bacillus xerosis 244

Bacterium coli commune; see *Bacillus coli communis.*

Bactericidal serum, substance, etc.; see Bacteriolysins.

Bacteriolysins 130

Absorption of, by bacteria, 136; composition of, 134; curative value of, 227, 231; endotoxins, action on, 136, 228; group reaction with, 135; immunity, relation of, to, 135; inactivation and reactivation of, 133, 134; nature and selective action of, 131; origin of, from body cells, 45, 138; properties, general, 130; prophylactic value of, 227; specificity of, 135; standardization of, 138; technic of testing, 139; therapeutic use of, 226; see Amboceptors and Complements.

Bacteriolysis and bacteriolysin 130

Bacteriolysis.

Group reaction, 153; mechanism of, 145; Pfeiffer's phenomenon, 131; similarity to hemolysis, 134; see Bacteriolysins.

Bacteriolytic enzymes, relation to immunity 61, 62

PAGE.
Bacteriotropic substances 227, 346, 369, 381
Balantidium coli, morphology, occurrence and pathogenicity 505, 506
Balantidium minutum 506
Benzol ring; use of, as an analogy in Ehrlich's theory 196.
Bile.
Bactericidal and antitoxic properties of, 40; immune agglutinins in, 95.
Biologic test for species; see Precipitins.
"Black Death"; see Plague.
"Blackwater fever" in malaria 477
Blastomycetic dermatitis; see Oidiomycosis.
Blastomycosis; see Oidiomycosis.
Blue pus 259
Bodo urinarius 508
Botulism 16, 256-259
Absorption of toxin, 258; antitoxin, 90, 259; immunity, 259; infected meats, 256, 257; phagocytosis, 258; prophylaxis, 259; susceptibility, 258; symptoms, 256; tissues affected by toxin, 258; see *Bacillus botulinus*.
Bovine pest 11
Bronchitis.
In epidemic cerebrospinal meningitis, 391; meningococcus in, 391; *Micrococcus catarrhalis* in, 384, 391; staphylococcus in, 377; streptococcus in, 354.
Capsulated bacilli 401, 402
Carbuncle, staphylococcus in 376, 377
Carcinoma, hereditary susceptibility to..... 18
Cell receptors; see Receptors.
Cercomonas intestinalis, morphology and pathogenicity of 506, 507
Chancroid; see soft chancre.
Chemicals in relation to antibody formation..... 198
Chemotaxis 43, 44, 177, 185
Chicken cholera, attenuation of microbe of 219
Chicken pox (varicella) 555, 556
Chicken typhus or chicken-pest 11
Cholera 10, 304-315
Accidental, in man, 313; agglutination reaction, 315; animals, susceptibility of, to, 309; anti-bodies, origin of, 190, 314; antitoxic serum, 315; bactericidal power of body fluids, 313; "cholera-carriers," 304, 313; diagnosis, bacteriologic, 315; epidemiology, 307, 308, 311, 312; experimental, in man, 313; gastric juice, protective action of, 313; geographic distribution of, 307, 308; immunity and susceptibility to, 56, 60, 190, 313, 314; infection atrium, 307; lesions, intestinal, 310; effect of leucotoxic serum on infections, 168; mechanism of intoxication, 310; mixed immunization in, 234, 314; phagocytosis, 188, 189, 313, 314; phagolysis, 188; prophylaxis, 218, 307, 311; serum properties in, 20, 97; serum therapy, 230, 314, 315; sources of infection and transmission, 307-309; vaccines and vaccination, 58, 312, 313; see *Vibrio choleræ*.
Cholesterin, neutralizing action on tetanolysin..... 91
Chromophages 179

PAGE.
Cladothrix, infections with 463
Clavelée (sheep-pox) 11, 554
Co-agglutinins 111
Cobra-lecithid 160, 206, 571
Cobra venom; see Venoms.
Coccidia, life cycle, morphology, spore formation and pathogenicity, 508, 509.
Coccidiosis 508, 509
Coccidium bigeminum 509
Coccidium cuniculi s. oviforme 509
Cocobacteria septica (Billroth) 349
Coley's mixture 362
Colle's law; see Syphilis.
Colloids 127, 128
Complement.
Absorption of, 145, 229; decrease of during disease, 230; diversion of, 157, 229, 230; isolation of, 140; lecithin as a, 160; multiplicity of, 153, 210; origin of, 138, 180; neutralization of, by salts, 91, 161; receptors of second order, 207; resistance to heat, 134; solutions of, 144; sources of, for bactericidal serums, 228, 229; specificity of, 152; structure of, 149; unicity, theory of, 210; see Cytase.
Complemintophilous haptophore; see Haptophore.
Complementoid 149, 209
Complementoid-Verstöpfung 150
Conjunctivitis.
B. influenzæ, in, 396, 397; diphtheritic, 237; meningococcus in, 391; pneumococcus in, 348, 349; staphylococcus in, 377.
Connective tissue, rôle of, in inflammation..... 5, 42, 46
Contact infection 3
Contagion and contagiousness 2
Contagious disease, definition 2
Copula of Müller, synonyms for 148
Cow pox 550, 554
Crotin 104, 264
Crystalloids, properties of 127
Culex fatigans 540
Culex pipiens, in transmission of malaria of birds.... 483
Curative injections 220
Cyclaster scarlatinalis 556
See Scarlet fever.
Cystomonas urinarius 508
Cytase 178, 181, 182
See Complement.
Cytoryctes variolæ s. vaccinæ 543
Conjugation, 544, 545; cytoplasmic stages, 544; life history of, 54-546; nuclear stages, 544; smallpox, in, 543; vaccinia, in, 545.
Cytotoxins (Cytolysins) 55, 62, 162-175
Activity, determination of, 164; amboceptors in, 165; antileucotoxin, 169; antinephrotoxin, 170; antispermotoxin, 166; autocytotoxins, 163, 174; autonephrotoxins, 169; autospermotoxin, 166; ciliated epithelium, cytotoxin for, 167; complements in, 165; for malignant tumors, 164; hepatotoxins, 171; infections, effect of leucotoxins on, 168; leucotoxin,

PAGE.
167; nephrotoxin, 169; neurotoxins, 171; origin, 180; of venoms, 265, 266; pancreotoxin, 173; specificity, lack of, 162; spermotoxin, 165; structure of, 165; syncytiotoxin, 171; technic of production, 164; thyrotoxin, 173; utility, theoretical, 162, 168.
Cytolysins; see Cytotoxins.
Dacryocystitis, pneumococcus in 348
Daphnia, phagocytosis of 183
Dengue fever 539, 541
Characteristics of, 546; contagiousness of, 540, 541; *Culex fatigans* in transmission of, 540; etiology, 540; occurrence, 539; "plasmeba" in, 540; recurrences and relapses, 541; susceptibility to, 541; transmission, 540.
Desmon 148
Deuterotoxin 83, 209
Diphtheria 10, 13, 20, 235-244
Agglutination reaction, 243; bacilli, localization of, 238; conjunctivitis, diphtheritic, 237; forms of, 237; immunity and susceptibility, 56, 61, 239, 240; infection atria, 237; latent, 237; leucocytes in, 240; mixed infections in, 14, 238, 186, 358; paralysis, influence of antitoxin on, 242; predisposing causes, 240; pneumonia in, 344; prophylaxis, 240, 241; pseudodiphtheria bacilli in, 243; recurrences, 56, 240; septic, 239; serum therapy, 225, 241; sources of infection, 236, 237; tissues injured by toxin, 238; transmission, 237; vulva, of, 237; see *Bacillus diphtheriæ*.
Diplococcus intracellularis meningitidis; see *Micrococcus meningitidis*.
Diplococcus pneumoniæ 336-349
Agglutination of, 347; alveolar abscess, in, 348; animals, susceptibility of, 339; antiserums, properties of, 346; conjunctivitis, 348, 349; dacryocystitis, 348; discovery of, 337; endotoxins, 339; enteritis, 348, 349; group agglutination, 348; immunization with, 345, 346, 347; influenza, in, 397; meningitis, 348, 349, 389; morphology, staining, and cultivation, 337, 338; neurotoxic strains of, 339; occurrence in blood, 343; occurrence, normal, 339; opsonins in phagocytosis of, 346; otitis media, in, 348, 349; peritonitis, in, 348, 349; phagocytosis of, 346; pneumonia, in, 337-348; pneumotoxin, 339, 346; pulmonary hemorrhage, in, 344; resemblance to streptococcus, 338; resistance, 338; rhinitis, in, 348; septicemia, 348; serpent ulcer, 348, 349; tuberculosis, in, 425; virulence, 338; virulence, increase of, 342; see Pneumonia.
Diplococcus (streptococcus) in rheumatic fever..... 359
Dourine; see Trypanosomiasis in animals.
Droplet infection 237
In diphtheria, 237; in influenza, 397; in tuberculosis, 415.
Drug habituation 22
Dust infection 237
In diphtheria, 237; in influenza, 397; in tuberculosis, 415; in typhoid fever, 272.

PAGE.
Dysentery, acute epidemic 8, 10, 288-294
Agglutination reaction, 294; antiserums, properties of, 293; bacilli, dissemination of, by stools, 292; bacilli, distribution of, in the body, 290; chronic, 288, 292; immunity and susceptibility, 60, 292, 293; incubation period, 288; institutions, occurrence in, 292; intestinal lesions in, 290; occurrence of, 288; predisposing causes of, 292; prophylaxis, 292; serum therapy, 293; summer diarrheas of infants, 289; transmission, 292; vaccination, 293; see *Bacillus dysenteriæ*.
Eclampsia, relation of syncytiotoxin to..... 171
Eczema, relation of staphylococcus to 376, 377
Eel serum, antitoxin for 90
Ehrlich's partial saturation method 80, 209
Ehrlich's "side-chain" theory. See "Side-chain" theory of Ehrlich.
Emboli, bacterial 4
Endocarditis.
Colon bacillus in, 302; gonorrheal, 386; pneumococcus in, 344, 348; staphylococcus in, 359, 377; streptococcus in, 354, 358, 359.
Encephalitis, in epidemic cerebrospinal meningitis.... 391
Endocomplement 159, 266
Endotheliotoxin, of venom 265
Endotoxins 330
Anthrax bacillus, 330; *Bacillus pyocyaneus*, 260, 261; bacteria containing, 226, 231; cholera vibrio, 309; diseases associated with, 269; dysentery bacillus, 291; failure of bactericidal serums to neutralize, 227; glanders bacillus, 453; of gonococcus, 385; leprosy bacillus, 450; liberation of, by bacteriolytic serums, 136, 228; meningococcus, 390; paratyphoid bacillus, 287; plague bacillus, 318; pneumococcus, 339; staphylococcus, 262, 373; streptococcus, 262, 353; tubercle bacillus, 411; typhoid bacillus, 271.
Enteritis.
Ameba coli in; see Amebic dysentery; *Balantidium coli* in, 505; *Cercomanas intestinalis* in, 506; colon bacillus in, 303; pneumococcus in, 348, 349; staphylococcus in, 377; streptococcus in, 354, 355, 357; *Trichomonas intestinalis* in, 507.
Enzymes, bacteriolytic, relation to immunity..... 61, 62
Enzymes, intracellular 176
Epilepsy, cytotoxin in 174
Epithelioma contagiosum of fowls 11
Epitoxoids 81
Erysipelas 355
Effect on tumors, 362; experimental production of, by streptococcus, 355; in course of tuberculosis, 356; recurrence of, 56; staphylococcus in, 355; streptococci in, 350, 354.
Etiology, infectious 7
Etiology, unknown 10, 510
Exhaustion, toxin of 174
Farcin du bœuf..... 463

PAGE.
Farcy, see Glanders 10
Fermentation, early studies on 27
Fibrin, mechanical value of in inflammation..... 45
Fixator, synonyms for 148
See Amboceptors.
Filaria perstans 487
Filaria sanguinis hominis 4
Fish, *B. enteritidis* in poisonous..... 297
Fish poisons, antitoxins for 90
Fleas, in the transmission of plague 320, 321
Flies, as carriers of typhoid fever..... 272
Fomites 3, 532, 539
Food-substances.
Fixation of, by amboceptors, 208; manner of union with cells, 197; non-formation of antibodies for, 199.
Foot and mouth disease 11, 12
Fowls, epithelioma contagiosum of 11
"Gambian Fever"; see Trypanosomatic Fever.
Gastric juice.
Protective rôle of, 39, 313, 329.
Gelatinase 371
German measles (Rötheln) 562
Glanders (Farcy) 10, 36, 452-458
Agglutination reaction, 458; animals, susceptibility of, 452; bacilli, distribution of, in the body, 454; connective tissue development in, 456; diagnosis, bacteriologic, 457; healing processes in, 456; immunity, 456; infection atria, 454, 455; mallein in diagnosis of, 457; organs involved, 456; phagocytosis, 456; serum therapy, 457; tissue reactions, 455; see *Bacillus mallei.*
Glossina palpalis in transmission of sleeping sickness.. 488
Gonococcus; see *Micrococcus gonorrheæ.*
Gonorrhea 10, 56, 384-388
Acute and chronic, 387, 388; complications of, 386, 387; immunity, 56, 387, 388; ophthalmia in, 386; phagocytosis, 385, 389; reinfection, 387, 388; superinfection, 388; susceptibility of different tissues to, 386; urethral changes, 387; see *Micrococcus gonorrheæ.*
Gonotoxin 386
Grass bacilli 444
Gregarina lindemanni; see Sarcosporidia.
Group agglutination 110, 113, 284, 287, 298, 348, 369
Gruber-Widal reaction; see Agglutination.
Hairs, phagocytosis of pigment by chromophages 179
Halteridium.
Impregnation of parasite, 468; in malaria of birds, 483.
Haptophores 79, 197, 199, 207
Haptophorous groups; see Haptophores.
Hauptagglutinins 111
Hay fever 262, 263
Antitoxin (pollantin), 90, 263; pollen as cause of, 262; toxin of, 262.
Hanging-drop preparation 98

PAGE.

Hemagglutinins.
Of plants, 103; of serums, 104; of venom, 265.
Hemoglobinuric fever, in malaria 477
Hemolysins.
Animal, 264, 268; bacterial, 202; cobra lecithid, 160, 266, 571; colloids as, 160; experimental value of, 141; from organ extracts, 180; immune, in serums, 62, 141; intraleucocytic, 180; normal, in serums, 54; pyocyanolysin, 260; serum hemolysins, structure of, 141; staphylococcus, see Staphylolysin; streptococcus, see Streptocolysin; tetanolysin, 249; venom, of, 265.
Hemolysis, see Hemolysins.
Hemolytic experiments.
Technic of, 141; value of, in study of immunity, 142
Hemorrhagic septicemia group of bacteria 316
Hemorrhagin 159, 265
Hemotoxins 67
Hepatotoxins 171
Heterologous serum 94
Homologous serum 94
"Horror autotoxicus" 174
Horsepox 554
Hydrophobia 10, 510-521
Animals, in, 513, 514; antiserum, properties of, 421; diagnosis, in dogs, 515, 516; extension through nerves, 517; fixed virus of, 513; immunity, character of, 521; immunization, mixed, 521; incubation period, 514, 515, 517, 518; micro-organisms found in, 510; Negri bodies, 510, 511; Pasteur treatment, 518, 521; prophylaxis of, 517, 521; specific (?) lesions, 516; street virus of, 512; transmission of, 514; toxin, question of, 511; vaccination, 29, 30, 519; vaccine, preparation of, 518, 519; virulence for man, 513, 517, 520; virulence, increase and decrease of, by passage, 513; virus, attenuation of, 58, 219, 511, 518-520; *virus de rue*, 513; virus, distribution and excretion of, 513; virus, filterability of, 12, 511; *virus fixe*, 513, 518; virus, resistance of, 512.
Ichthyosismus 256
Ichthyotoxin 268
Immunity.
Absolute, 21; acquired, 18, 56-64; active, 21, 56, 59; antibacterial, 19, 47, 48, 49, 211; antitoxic, 19, 49, 50, 210; definition of, 17; in families, 18; leucocytes, relation to; see Phagocytosis; natural, 18, 35-55; early theories of, 24; passive, 21, 60; relative, 21; theories of, 30-34; types of, 22; see Antitoxins, Bacteriolysins, Phagocytosis and the individual diseases.
Immunization.
Active, as curative measure, 220; active, for prophylaxis, 232; classification of methods, 218; choice of animals for, 229; mixed, 220, 234; passive, as cura-

PAGE.
tive measure, 220; passive, in prophylaxis, 220; with tissue cells, 164; with toxins, 33.
Impetigo contagiosa.
Staphylococcus in, 354; streptococcus in, 357.
Incubation period 210
Infection.
"Air borne," 3; atrium of, 3, 35, 39; contact, by, 3; mixed, 11, 14, 15, 113; see individual diseases; "water borne," 3; infectious agents, classification of, 6.
Infectiousness and contagiousness 2
Infestation 2
Inflammation.
Antagonism of, to infections, 46; chemotaxis, 43; connective tissue, inflammatory rôle of, 41, 46; fibrin, influence of, 45; injurious effects of, 41, 42; leucocytes in, 43-45; nature of, 41; organization in, 45; phagocytosis in, 43-45; plasma, influence of, 45; relation of, to virulence of bacteria, 42, 43; rôle of, in immunity and resistance, 41; variations in intensity, 42-44.
Influenza 10, 393-399
Conjunctivitis in, 37, 397; chronic, 397; contagiousness of, 393; epidemics of, 393; immunity, 398; infection atrium, 397; intestinal, 396; intoxication, 396; meningitis in, 396, 397; mixed and secondary infections in, 397; otitis media in, 396, 397; peritonitis in, 396, 397; phagocytosis in, 396; pneumonia during, 344, 396; prophylaxis, 398; recurrence of, 56, 398; susceptibility, 398; transmission of, 397; tuberculosis during, 397; see *Bacillus influenzæ*.
Injuries, mechanical and toxic, by bacteria 4
"Intestinal group" of bacteria 270
Intoxications, infectious 15
Isoagglutinins 104
Isoprecipitins 121
Lactoserum 120, 125
Lamblia intestinalis 508
"Leistungskern" 86, 195
Lecithin as endocomplement 159
"Leprolin" 450
Leprosy 10, 445-452
Animals, insusceptibility of, to, 447; contagiousness of, 446; distribution of bacilli in the body, 449; extension and occurrence of, 445; fish, relation of to, 448; infection atria, 448; intercurrent infections, 450; phagocytosis in, 449, 451; potassium iodid in treatment of, 450; prophylaxis, 451; protective factors in, 451; serum therapy of, 452; spontaneous disappearance of, 450; susceptibility to, 451; transmission of, 448; tubercular, 450; see *Bacillus lepræ*.
Leptothrix, infections by 463
Leptothrix baccalis 463
Leptothrix vaginalis 463

PAGE.
Leucocidin 67, 202, 373, 380, 382
Antitoxin for, 372; influence on phagocytosis, 372.
Leucocytes.
Absorption of toxins by, 191; complement in, 154; formation of precipitins by, 121; immunity, relation to, 176-194; in inflammations, 43; phagocytic properties of, 43, 44, 45; see also individual diseases; see Phagocytosis.
Leucocytic exudates, bactericidal action of..... 378, 379
"Loop," standard 101
Leucotoxic serum 167, 168
Leucotoxin; see Leucotoxic Serum.
"Lumpy jaw"; see Actinomycosis.
Lupus, influence of streptococcus on..... 362
Lymphangitis, streptococcus in 354-356
Lymphatotoxin; see Leucotoxic Serum.
Macrocytase 178
Macroparasites 6
Macrophages 44, 168, 178, 184
Madura foot; see Mycetoma.
Mal de cederas; see Trypanosomiasis in animals.
Malaria 468-483
Æstivo-autumnal, 469; æstivo-autumnal, parasite of; see *Plasmodium præcox;* anemia in, 474; "blackwater fever" in, 477; cachexia in, 476; cerebral symptoms, 477; epidemiology of, 477; etiology of, 468; fever, relation of to developmental cycles of parasites, 474; hemoglobinuric fever in, 477; immunity, acquired, 481, 482; incubation period, 473; intestinal symptoms, 477; melanemia in, 474; methylene blue in, 474; mixed infections, 476; mosquitoes, transmission by, 468; neuralgia in, 476; parasites, localization of, 477; prophylaxis of, 480, 481; quartan, 469; quartan, parasite of; see *Plasmodium malariæ;* quinin in prophylaxis and treatment of, 476, 477, 481; quotidian, 475; parasites, localization of, 473, 474, 477; relation of clinical symptoms to developmental cycles of parasites, 473; susceptibility to, 481; tertian, 469; tertian, parasite of; see *Plasmodium vivax;* toxins, 474, 475; transmission; see Anopheles; see Plasmodium of malaria.
Malaria of birds, halteridium in; proteosoma in 483
Malignant pustule; see "Anthrax."
Mallein 220, 454, 457
Malta Fever 333-335
Accidental infections, 335; agglutination reaction in, 334; difference from typhoid fever, 334; distribution of bacillus in body, 334, 335; immunity, 335; occurrence, 333; serum, properties of, 334; serum therapy, 335; transmission, 335; see *Bacillus melitensis.*
Measles 559-562
Complications and sequelæ, 560; contagiousness of, 559; immunity and susceptibility, 561; leprosy, influence on, 450; leucocytes in, 561; *Micrococcus*

PAGE.
catarrhalis in, 384; micro-organisms in, 559; prophylaxis, 560; racial immunization, 561; resistance of virus, 560; recurrences, 561; serum therapy, 561; virus, distribution of, 559, 560.
Meat poisoning.
Bacillus botulinus in, 256; *Bacillus enteritidis* in, 294-298; *Bacillus paratyphosus* in, 285; relation of ptomaines to, 295.
Mechanical and toxic injuries 4
Mediterranean fever, see Malta fever.
Meningitis.
B. pneumoniæ in, 402; colon bacillus in, 302; in influenza, 396; micro-organisms causing, 388, 189; pneumococcus in, 344, 348, 349; secondary, 356, 357; streptococcus in, 354, 356, 357, 358; tuberculous, 420.
Meningitis, epidemic cerebrospinal 388-393
Agglutination test, 393; cerebrospinal character of, 391; complications, 391; contagiousness of, 392; immunity, acquired, 392; lumbar puncture for diagnosis, 391; metastatic infections, 391; mixed and secondary infections in, 391; prophylaxis of, 392; secondary to rhinitis, 390; serum, properties of, 393; susceptibility to, 392; transmission of, 392; see *Micrococcus meningitidis.*
Meningococcus; see *Micrococcus meningitidis.*
Metchnikoff's theory; see Phagocytosis.
Methylene blue, effect of, on malarial parasites 474
Microbic specificity 27
Micrococcus catarrhalis 383, 384
Animals, susceptibility of, to, 384; bronchitis, in, 384, 391; measles, in, 384; occurrence in respiratory passages, 383; occurrence under normal conditions, 384; pneumonia, in, 337, 344, 384, 392; resemblance to meningococcus, 392; scarlet fever, in, 384; whooping-cough, in, 383.
Micrococcus gonorrheæ (gonococcus) 383
Antiserum for, 388; cultivation of, 384, 385; discovery of, 384; endotoxin of, 385; gonotoxin, 386; immunization with, 388; infections with, 384, 388; morphology, 384; phagocytosis of, 385, 387; resistance of, 385; toxin, soluble, 388; see Gonorrhea.
Micrococcus hematodes 374
Micrococcus melitensis, see *Bacillus melitensis.*
Micrococcus meningitidis (*Diplococcus intracellularis meningitidis,* or the meningococcus) 10, 388-393
Agglutination of, 393; animals, susceptibility of, 390; antiserum, properties of, 392, 393; bronchitis, in, 391; conjunctivitis, in, 391; cultivation, 390; discovery, 389; endotoxin, 390; excretion of, 392; immunization with, 393; morphology, 390; pneumonia, in, 391; resemblance to gonococcus, 389; resemblance to *Micrococcus catarrhalis,* 392; resistance, 390; rhinitis, in, 391; virulence, 390; see Meningitis, epidemic cerebrospinal.
Microbic specificity 27
Microcytase 178-189

PAGE.

Micro-organisms.
Early belief in, 25; recognition of, 26; ultramicroscopic, 6.
Microparasites 6
Microphages 44, 178, 184, 190
Microsporon septicum (Klebs) 349
Milk bacilli 444
Mitagglutinin 111
"Monadinin" of Klebs 359
Mucor 467
Mumps (epidemic parotitis) 566
Mycetoma 462
Nagana; see Trypanosomiasis in animals 493
Natural immunity; see Immunity.
"Negative phase" following vaccinations 233
Negri bodies; see Hydrophobia.
Nephrotoxin 169, 170
Neuronophages 179
Neurotoxin of serums 171
Neurotoxin of venom 67, 265
Oidia 6, 10
Oidiomycosis 463-467
In animals, 467; cutaneous, 463; infection atria, 465; organisms of, 464; resemblance to tuberculosis, 465; systemic, 464; thrush, 466.
Oidium 464
Agglutination, immunization and phagocytosis, 467.
Oidium albicans 466
Oidium coccidioides 465
Old age, Metchinkoff's theory of 168
Ophthalmia.
Cytotoxins in, 173; gonorrheal, 386.
Opsonic index 382
Opsonins 61, 193, 227, 369
Action of salts on, 194; in acquired immunity, 6; see Phagocytosis in different diseases.
Opsonoid 194
Otitis media.
B. influenzæ in, 396, 397; pneumococcus in, 348; staphylococcus in, 377; streptococcus in, 358; tuberculous, 420.
Oxytuberculin 413
Ozena 402
Pancreatic juice, action on toxins 40
Pancreotoxin 173
"Paracolon" bacilli 285
Parasites, pathogenic 1, 6
Parasitism 1
Parotitis, epidemic; see Mumps.
Passive immunity; see Immunity.
Paratyphoid fever 284-288
Agglutination reaction in, 287, 288; blood cultures in, 288; characteristics of the disease, 286; endotoxin of bacilli, 287; epidemiology of, 285; as meat poisoning, 285; occurrence of bacilli in the body, 286; properties of serum, 287; prophylaxis, 287; transmission, 285, 286; see *Bacillus paratyphosus.*
Passage 58

PAGE.
Pasteur treatment; see Hydrophobia.
Pathogenesis 4, 5
Peripneumonia of cattle 11, 12
Periostitis albuminosa 377
Periostitis, staphylococcus in 377
Peritonitis.
Colon bacillus in, 303; by influenza bacillus, 396, 397; pneumococcus in, 348, 349; staphylococcus in, 377; streptococcus in, 354, 357; tuberculous, 420.
Pertussis; see Whooping-cough.
Pfeiffer's phenomenon 96, 131, 132
In identifying the vibrio of cholera, 305; rôle of leucocytes in, 188.
Phagocytic (Metchnikoff's) theory of immunity 176, 194
Comparison of, with the side-chain theory of Ehrlich, 212, 215; see Phagocytosis.
Phagocytosis 31, 44, 176-194, 215
In active immunity, 59, 60, 192, 193; chemotaxis in, 177, 185; fixators, influence of, 190; in inflammations, 43; intestinal, 41, 177; intravascular, 188; leucocidin, influence of, 372; in nutrition, 172; opsonins,, rôle of, 193; in passive immunity, 60, 192, 193; relation of to virulence of bacteria, 184, 187; in resorption, 178; serum, influence of, 187, 193; in vitro, 49, 193; see under the individual microorganisms and diseases; see Opsonins.
Phagolysis 181, 182, 188, 190
Phallin 264
Phrynolysin 268
Phytoprecipitins 120
Plagiomonas urinaria 508
Plague 10, 315-327
Agglutination reaction, 326, 327; animals, susceptibility of, 319; contagiousness, 322; diagnosis, bacteriologic, 323; dissemination of bacillus by urine, feces, sputum, 322; epidemiology, 320, 323; foci of, 320; immunity, 56, 60, 324, 325; infection atria, 322; mixed immunization in, 234, 324; mixed infection in, 323; occurrence, 315; houses, 321; prophylaxis, 323; in rats, 320; serum therapy, 325, 326; transmission by fleas, 320, 321; transmission from rat to man, 320, 321; vaccination, 58, 218, 323; vaccines, 323, 324; see *Bacillus pestis.*
Plasmin of Buchner 68, 219
Plasmodia of malaria 4, 10, 468
Anopheles mosquito as host of, 9, 469; asexual cycle, 469; development in anopheles, 470; development in man, 469; discovery of, 468; flagella of, 468; macrogamete, 470; merozoites, 470; methylene blue, effect of, 474; microgamete, 471; microgametocyte, 470; oöcyst, 471; oökinet, 471; schizogony, 470; sexual cycle, 470; species of, 469; spermatozoites, 468; sporocyte, 470; sporogony, 471; sporozoites, 471; see Malaria.
Plasmodium malariæ 469
Relation to clinical symptoms, 473; sexual and asexual cycles of, 472; virulence, 474.
Plasmodium præcox 469

PAGE.
Relation to clinical symptoms, 473; sexual and asexual cycles of, 472; virulence, 473.
Plasmodium vivax 469
Asexual cycle of, 469, 470; relation of to clinical symptoms, 473; sexual cycle of, 470, 471; virulence 474
Pneumococcus; see *Diplococcus pneumoniæ.*
Pneumonia 336-348
Agglutination reaction, 347; *B. pneumoniæ* in, 402 bacteria causing, 336, 337; causes, predisposing, 343; complications, 343, 344, 348; contagiousness, 342; immunity and susceptibility, 56, 345; infection atrium and method of infection, 340; influenza bacillus in, 396; leucocytes, 345; metastatic infections, 348; phagocytosis, 345; meningococcus in, 391; *Micrococcus catarrhalis* in, 384, 392; mixed infections in, 344; polyvalent serum for, 347; prophylaxis, 344; recurrences, 345; Roemer's serum, 347; serum properties, 345; serum therapy, 346, 347; staphylococcus in, 377; streptococcus in, 354, 355, 356; vaccination, 345; see *Diplococcus pneumoniæ* and other bacteria enumerated on page 336.
Pneumotoxin 339
Pollantin 263
Pollens.
As cause of hay fever, 262; antitoxin for, 263.
Polyceptors 155
Polyvalent serums.
For pneumococcus, 347; for staphylococcus, 383; for streptococcus, 232, 366.
"Positive phase" following vaccination 233
Postmortem invasion 302
Precipitate 120, 124, 125
Precipitation reaction 106, 119
Agglutination, relation to, 117; as clinical reaction, 119; with colloids and electrolytes, 128, 129; forensic use of, 63, 125; precipitation, group reaction, 125, 126; meats, differentiation of, 127; physical chemistry in the study of, 129; specific inhibition, 122; technic, 126; use of in studying reactions of immunity, 206.
Precipitins 119-129
Antiprecipitins, 123; autoprecipitins, 121; bacterial, 63, 119, 233, 314; formation of, 121; isoprecipitins, 121; lactoserum, 120; phytoprecipitins, 120; resistance to ferments, heat, etc., 122; serum precipitins, immune, 63; serum precipitins, normal, 55; structure of, 122; zoöprecipitins, 120.
Precipitogens 120
Precipitoids 122
Pregnancy, serum diagnosis 172
Preparator 148
Proagglutinoids 109
Prophylactic injections, classification of methods 218
Protective inoculation; see Vaccination.
Proteins 220
Proteosoma in malaria of birds 483
Prototoxin 83, 209
Protoxoids 82, 209

PAGE.
Protozoa, infections with....468, 509
Pseudodiphtheria bacilli 243, 244, 425
Pseudoinfluenza bacilli 394
Pseudotuberculosis of animals.... 444
Ptomains in meat.... 295
Pyocyanase 61, 260
Pyocyanin 260
Pyocyanolysin 260
Pyroplasma bovis; see Texas fever.
Pyroplasma hominis; see Spotted fever
Pyroplasmosis; see Spotted fever and Texas fever.
Rabies; see Hydrophobia.
Radium, effect on venom 267
Rats in epidemics of plague 320, 321
Rattlesnake venom.
  Antiserum for, 267; immunization with, 219.
Ray fungus; see Actinomyces.... 320, 321
Receptors.
  Bacterial, 152; function of, 52, 87, 88; immunity, relation to, 199; loss of, as cause of immunity, 240; multiplicity of, 86, 208; new formation of, 199, 205, 207; nutrition, relation to, 195; of first order, 89, 200, 207; of second order, 114 203, 207, 209; of the third order, 150, 203, 207; synonym for side chain, 197; tetanophile receptors of nervous tissue, 252; types of, 207; see different antibodies.
Relapsing fever 10, 403-406
  Agglutination test, 406; immunity and susceptibility, 404, 406; organism of; see *Spirocheta obermeieri;* phagocytosis in, 405; prophylaxis, 404; serum properties, 405, 406; serum therapy, 406; transmission of by bedbugs, 404.
Resistance, natural; see Immunity, natural.
Resorption.
  Of foreign cells, 179; of native cells, 178.
Rheumatic fever 359, 360
  Agonal invasions in, 359; antistreptococcus serum in, 368; bacillus of Achalme in, 359; diplococcus (streptococcus) in, 360; experimental, 359, 360; micro-organisms found in lesions, 359; staphylococcus in, 359; streptococcus in, 354, 359, 360; *Zymotosis translucens,* 359.
Rhinitis.
  Meningococcus in, 391; pneumococcus in, 348; primary to meningitis, 390; staphylococcus in, 377; *Rhinitis fibrinosa,* 237.
Rhinoscleroma 402
Ricin 6, 264
  Antiricin, 63; Ehrlich's use of in studying nature of antitoxic action, 201, 202; hemagglutinin in, 104.
Robin 264
Rötheln, see German measles.
Saccharomycosis hominis.... 464
Salamander poison, antitoxin for.... 90
Saprophytes 1
  In tetanus 248
Sarcocystis hominis; see Sarcosporidia.

PAGE.

Sarcosporidia.
Morphology, occurrence and proliferation, 504, 505; *Sarcocystis hominis*, 505.
Scarlatina; see Scarlet fever.
Scarlet fever (scarlatina) 556-559
Agglutination of streptococci by serum, 369, 370; contagiousness, 557; *Cyclaster scarlatinalis* in, 556; *Diplococcus scarlatinæ*, 11; leucocytes in, 558; *Micrococcus catarrhalis*, 384; micro-organisms in, 557; prophylaxis, 557; protozoa in, 10, 556; resistance of virus, 557; serum therapy, 558, 559; streptococcus in, 14, 15, 186, 354, 355, 358, 361, 556; *Streptococcus scarlatinæ*, 361; immunity and susceptibility, 56, 558; serum therapy (antistreptococcus), 366, 368, 558; transmission, 557.
Scorpion, toxin and antitoxin 90, 268
Sensitization 145
Serpent ulcer.
Pneumococcus in, 348, 349; treatment with antipneumococcus serum (Roemer), 349.
Serums, purity of 76, 221
Serum therapy, principles of 218-234
Antitoxins, 221, 226; bactericidal serums, 226, 232; classification of methods, 218; curative injections, 220, 223, 227; prophylactic injections, 218, 226, 227; see also under the different diseases.
Sheep-pox; see Clavelée.
Side-chain theory of Ehrlich 195-217
Amboceptor formation, 150; agglutinin formation, 114; antitoxin formation, 86, 89; applied to cell nutrition, 195; as applied to immunity, 199; chemical processes, 78, 200, 205, 208; complements, 153, 210; essential tenets of, 200; haptophores, 197; "*Leistungskern*," 195; Metchnikoff's phagocytic theory, comparison with, 212, 217; precipitin formation, 121; receptor proliferation, 205; receptors, types of, 207; side chains, 195.
Sleeping sickness 486-490
Anatomic lesions of, 489; bacteria in, 487; occurrence, 486, 487; symptoms of, 488, 489; transmission of, 488; Trypanosomatic fever, relation to, 489, 490; trypanosomes in, 487; see Trypanosomiasis.
Smallpox 541-555
Bacteria in, 542, 548; conversion into vaccinia, 541, 542; cyclic nature of symptoms, 547; *Cytoryctes variolæ s. vacciniæ*, 543; dissemination of virus, 546; etiology, 542; fetal, 548; immunity and susceptibility, 56, 554; incubation period, 547; infection atrium, 546; inoculation into calves, 541; Jennerization, 549; leucocytes in, 554; mixed (secondary) infections, 15, 548; nonfiltrability of virus, 542; prophylaxis, 548; protozoön-like bodies in, 11, 542; relation to vaccinia, 57, 541; revaccination, 552, 553; serum properties, 554; transmission, 546; vaccination, 548; vaccine, contaminations of, 551, 552; vaccine, durability of, 551; virulence, variations in, 547; virus, distribution in the body, 547.

PAGE.
Smallpox and vaccinia....541, 555
Smegma bacilli 443
Snake bites 264, 268
Soft chancre or chancroid 399-401
In animals, 400; bacillus of, 400; immunity, 401; independence, 399; infectiousness of, 399; phagocytosis, 400, 401.
Specific infections 9
Spermophilus columbianus as host of *Pyroplasma hominis* 498
Spermotoxin 165, 166
Spider poison, antitoxin for.... 90
Spirocheta obermeieri 10, 403
Agglutinins for, 406; animals, susceptibility of, 404; antiserums, properties of, 405; distribution in the body, 403; morphology, 403; occurrence in bedbugs, 404; phagocytosis of, 405; see Relapsing fever.
Spirocheta pallida (see syphilis).... 525, 526
"Spotted fever" 497, 498
Pyroplasma hominis in.... 497
Staphylococcus pyogenes, or staphylococcus 370-383
Agglutination, 92, 382; amyloid degeneration, 375; animals, susceptibility of, 375, 376; antiserums, properties, 380; bactericidal action of leucocytic exudates, 378, 379; bacteriolysin, 379, 380; discovery, 350; endotoxin, 373; ferments of, 371; hemolytic action, 5, 371, 372; immunity, 186, 379, 380; leucocidin, 372, 373, 380, 382; leucocytes in infections, 378, 379; leucotactic substance, 375; mixed infections, 378; morphology, 370; necrotizing substance, 375; pathogenic powers, 37, 336, 344, 354, 359, 376, 378, 389; opsonins in phagocytosis of, 382; phagocytosis, 378, 379, 380, 382; pigment formation, 374; polyvalent serum, 383; resistance, 374, 375; staphylolysin, 271, 372, 373, 375, 380; symbiosis with *Ameba coli*, 502; symbiosis with *B. influenzæ*, 394; toxicity of culture filtrates, 372, 373; toxin, soluble, 375; vaccination against, 381; varieties, 373, 374; virulence, 376.
Staphylolysin; see Staphylococcus.
Stegomyia fasciata and its relation to yellow fever.... 531
Streptococci in scarlet fever.....14, 354, 355, 358, 361
Streptococcus brevis 350
Streptococcus erysipelatis 350
Streptococcus longus 350
Streptococcus mucosus capsulatus 351
Streptococcus pyogenes....349-370
Agglutination, 92, 369, 370; animals, susceptibility of, 352; antagonism for *B. anthracis*, 329, 362; for *B. tuberculosis*, 356; antistreptococcus serum, properties of, 362, 368; antistreptocolysin, 353; bacteriotropic substances, 369; Coley's mixture, 362; cultivation, 351; in diphtheria, 238, 358; discovery, 350; endotoxin, 353; erysipelas, 350, 354, 355; immunity, 186, 363, 364, 365; infection atria, 358; infections, miscellaneous, 354, 362; in influenza, 397; leucocytes and leucocytosis, 363, 364; lupus, influence on, 262; in meningitis, 354, 389; in milk, 357; morphology, 350; opsonins, 369; pha-

PAGE.

gocytosis, 363, 364; in pneumonia, 336, 344, 354, 355, 356; resemblance to pneumococcus, 338; resistance, 351, 352; in rheumatic fever, 354, 359; in scarlet fever, 14, 354, 355, 358, 366; serum therapy, 367-369; serums, univalent and polyvalent, 365; streptocolysin, 353, 354; symbiosis with *B. influenzæ*, 394; tissue reactions, 42; toxic properties, 353, 354, 362; toxin for erythrocytes; see Streptocolysin; toxin for leucocytes, 354; in tuberculosis, 355, 356, 424, 425; tumors, influence on, 362; in typhoid fever, 275; unity, question of, 365; varieties, 350; virulence, 352.

Streptococcus scarlatinæ 361
Streptocolysin 353, 354
Streptothrix, infections with 463
Streptothrix maduræ 462
Substance sensibilisatrice 148, 217
Summer diarrheas; see Dysentery, acute epidemic.
Surra; see Trypanosomiasis in animals..... 494
Susceptibility 18, 53, 54
See the individual diseases.
Symptomatic anthrax.
Antitoxin, 90; vaccination against, 219.
Syncytiolysin 171
Synonyms 217
Syntoxoids 83, 209
Syphilis 10, 522-529
Animals, non-susceptibility of, 522; bacillus of De Lisle and Julien, 522; bacillus of Joseph and Piorkowski, 522; bacillus of Lustgarten, 522; Colle's law, 528; immunity and susceptibility, 54, 527; inheritance, 527; micro-organisms found in, 522; monkeys, transmission to, 12, 522; reinfection, 527; *Spirocheta pallida* in, 525; transmission, 526; virulence, variations in, 527; virus, distribution of, 526; virus, non-filterability of, 526.
Tetanolysin 249, 250
Antitetanolysin, 223; neutralization by cholesterin, 91.
Tetanospasmin 249, 250
Tetanus 10, 244-256
Agglutination reaction, 256; animals, susceptibility of, 51; cerebral, 251; dirt and necrotic tissue, influence of, 247; dolorosa, 251; excretion of toxin, 250; Fourth of July, 248, 252; "head tetanus," 251; in horses, 252; immunity and susceptibility, 18, 60, 185, 249, 251; "idiopathic," 248; incubation period, 249; leucocytes, in absorption of toxin, 250; local, 251; lumbar puncture, 255; mixed infections, 14, 248, 249; nervous tissue, in fixation and transport of toxin, 250; non-contagiousness of, 3; occurrence of bacillus in the body, 249; occurrence of toxin in the body, 250; pathogenesis, 250, 251; phagocytosis in, 185, 248; puerperal, 252; rheumaticus, 248; seasons in relation to prevalence, 248; serum therapy and prophylaxis, 224, 253-255; toxin (see *B. tetani*, toxin of); treatment of wounds, 252; Wassermann's experiment, 250; wounds favoring development of, 247; see *Bacillus tetani*.

PAGE.
Texas fever 499
Thrush 466
Organisms of, 466; susceptibility to, 467; systemic infections, 466.
Thyrotoxin 173
"Tick fever"; see "Spotted fever."
Timothy bacillus 444
Toxins.
Animal, 6, 264-268; attenuation of, 70; bacterial, 6, 235; see individual bacteria; chemotaxis, influence on, 185, 354, 372; crotin, 264; degenerative changes in, 80, 209; discovery of, 33; effects of, 4, 5; endotoxins, 6, 20, 68; see individual bacteria; gastric juice, destructive action, 39; haptophores of, 79, 209; see side-chain theory; immunization with, 69, 70, 220; incubation period of, 65, 251; intracellular; see Endotoxins; leucocidin, 372; leucocytes in absorption of, 45, 191; modifications by age, 85; neutralization by antitoxins, 48, 78, 200; pancreatic juice, destructive action, 40; phallin, 264; of pollens, 262; precipitation of, 66; preparation, 66; properties of, 65, 208; as receptors of, second order, 207; ricin, 264; robin, 264; secondary or adventitious, 67; selective action of, 5; toxin spectrum, 81, 209; standardization of, 72; staphylolysin, 371; structure, 78; toxophores, 80, 209; see side-chain theory; union with tissue cells, 66, 87, 200, 204, 224, 222, 252; vegetable, 6, 262-264; see individual micro-organisms.
Toxoids 80-83, 209
Toxon, ... 82, 209, 242
Toxophorous group, toxophore 80, 207
Trichomonas intestinalis, morphology and pathogenicity of 507, 508
Trichomonas vaginalis 507
Trichophyton 467
Tritotoxin 83, 209
Trypanosoma 483
Agglutination of, 485; cultivation of, 492, 494; morphology of, 484; multiplication of, 485; rosette formation by, 485; sleeping sickness, 487; species of, 484, 485, 490; trypanosomatic fever, 486.
Trypanosomatic fever 485, 486
Sleeping sickness, relation to, 489, 490; symptoms of, 486; trypanosomes in, 486.
Trypanosomiasis 483-497
Agglutination reactions, 497; immunity and susceptibility, 495; in man, 485-490; parasites, occurrence of in the blood, 491; serum therapy of, 496; "trypanroth" in treatment of, 496; vaccination in, 496; see Sleeping sickness and Trypanosomatic fever.
Trypanosomiasis in animals 490-495
Dourine, 495; horses, cattle and mules, 493-495; *mal de cederas*, 495; nagana, 493; in rats, 491; surra, 494; symptomatology, 491.

PAGE.
"Trypanroth" in treatment of trypanosomiasis 496
Tsetse flies, in transmission of trypanosomiasis; see Trypanosomiasis.
Tuberculin of Koch and others.... 219, 220, 412, 413, 414
Dangers, errors and limitations in use, 434, 435; diagnostic use of, 432-436, 442; disturbances caused by, 433; immunization with, 411, 431, 432; preparation of, 412; principles of action, 437; specificity of, 434, 435; standardization of, 412, 414; therapy, 436, 437.
Tuberculocidin 413
Tuberculosis 10, 407-443
Agglutination as an index of immunity to, 437; agglutination reaction, 438, 440; amyloid degeneration in, 424; "anatomic tubercle," 419; in animals, 427, 441; avian, 442; bovine, 441; bovine, relation of, to human, 415-417; congenital, 417; disinfection in, 426; dissemination by means of phagocytes, 421; "droplet infection," 418; "dust infection," 418; healing, spontaneous, 420; heredity in, 429; immunity and susceptibility, 18, 19, 61, 427, 430-432, 437; immunization, mixed, 439; infection atria, 418, 420; infectiveness of, 407; latent, 418; lupus vulgaris, 419; metastases in, 420; miliary, 421; mixed infections in, 424; organs attacked, 419-421; phagocytosis, 421, 422; pneumonia during, 344; predisposing factors to, 428, 429; primary and secondary, 421; prophylaxis, 425, 427; pulmonary, 418; serum therapy, 425, 438; streptococcus in, 355, 356; tissue changes in, 5, 422-425; tuberculin in diagnosis, 432-436; ulcerative, 419; vaccination against, 439.
Tumors, influence of streptococcus on 362
Turtle poison, antitoxin for 90
Typhoid fever 10, 269-284
Agglutination reaction, 7, 95, 283, 284; antibodies, origin of, 190; bacillemia, 272, 273; bacilli, distribution in the body, 273, 274; blood cultures for diagnosis, 273, 284; "dust" infection, 272; epidemiology of, 271; flies as carriers, 272; immunity and susceptibility, 56, 60, 61, 275; infection atrium, 272; leucocytes, 274, 277; leucotoxin in experimental infections, 168; mixed immunization, 234, 282; mixed infections in, 275; serum properties, 20 prophylaxis, 278; serum prophylaxis, 279; serum therapy, 230, 282; therapy, active immunization, 283; therapy of Jez, 283; vaccines and vaccination, 58, 218, 279, 282; see *B. typhosus.*
Typhus fever 10, 538-541
Conditions for development, 538; contagiousness, 539; fomites, 539; micro-organisms in, 538; occurrence, 538; prophylaxis, 539; pyroplasma (?) in, 538; serum therapy, 539; transmission, 539.
Ultramicroscopic micro-organisms 12
Uncinaria duodenalis 4
Undulant fever; see Malta fever.
Univalent serums 366
Urease 90

PAGE.
Vaccination 28, 57, 218-220, 232-234
Antibodies produced by, 233; duration of resistance caused by, 232; incubation period, relation to, 234; see Smallpox and Hydrophobia; opsonins, increase of, 234; "positive" and "negative phases," 233; substances used for, 218-220; see the individual diseases.
Vaccines 218-220, 232-234
See the individual diseases.
Vaccinia; see Smallpox and Vaccinia.
Varicella; see Chickenpox.
Variola inoculata 546, 591
Variola; see Smallpox.
Venoms 24, 66, 78, 85, 158-160, 264-268
Amboceptors and complements, 159, 266; antivenins, 63, 267, 268; character of, from different snakes, 265; cobra-lecithid, 160, 266; cytotoxins of, 265; endocomplements for, 159, 266; endotheliotoxin of, 265; ferments of, 266; hemagglutinins of, 158, 265; hemolysin of, 158, 265; hemorrhagin, 159, 265; incubation period, 66, 267; lecithin as complement, 159, 266; neurotoxin of, 158, 265; radium, effect of, 267; structure of cytotoxins of, 266; toxins of, 158; toxoids of, 265.
Vibrio choleræ.
Acquired immunity to, 186; action of gastric juice on, 39; active immunity, formation of specific precipitin in, 314; agglutination of, by normal serum, 92; agglutination of, 315; agglutinins, 314; attenuation of, 57; autolytic products, vaccination with, 313; discovery, 304; endotoxin of, 309; identification of, by agglutination reaction and by Pfeiffer experiment, 305; in Pfeiffer's phenomenon, 131; in stools of convalescents, 307; location in infected body, 310; morphology, staining properties and cultivation of, 304, 305; non-neutralization of endotoxin of, by its specific bactericidal serum, 137; occurrence of water, 307; resistance and viability of, 306; see Cholera; symbiosis with *Ameba coli*, 502; soluble toxin (?) 310; specificity of, 9; toxicity of culture filtrates, 309; toxicity of killed cultures, 309.
Vibrio metchnikovi..... 33
Virulence.
Increase of, in the presence of other micro-organisms, 14; influence of, on inflammatory reaction, 42; relation of, to phagocytosis, 184; see different micro-organisms.
Wasp poison, antitoxin for 90
Whooping cough (pertussis) 562-566
Contagiousness, 564, 565; cultural characteristics and pathogenicity of the influenza-like bacillus, 562 563; immunity and susceptibility, 565; influenza-like bacillus in, 562; influenza-like bacillus, relation to whooping cough, 564; *Micrococcus catarrhalis* in, 383; micro-organisms in, 562; prophylaxis, 565; pseudo-influenza bacilli in, 395; serum therapy, 565; virus, dissemination of, 564.

PAGE.
"Water-borne" epidemics; cholera, dysentery, typhoid. 271, 292, 308
Welch, hypothesis of 567
Widal reaction 92, 111
See Agglutination.
Wool-sorters' disease; see Anthrax.
Wright's method of vaccination.
Staphylococcus infections, 381; typhoid fever, 279, 280.
Yellow fever 10, 529-538
Acclimatization, question of, 537; altitude and moisture, relation to, 533; *Bacillus icteroides* in, 530, 531; cold, relation to, 533; epidemiology, 533; fomites, 532, 533; immunity acquired, 532, 538; importation by ships, 536; incubation period, 532; mosquito theory of, 530; see also *Stegomyia fasciata;* non-contagiousness of, 533; occurrence, 529; prophylaxis and quarantine of, 533, 534, 536, 537; virus, filterability of, 532; virus, resistance of, 536; serum therapy, 538; susceptibility to, 537.
Zone, contagious 2, 3
Zoöprecipitins 120
Zoötoxins 268
Zwischenkörper, synonyms for 148
Zymotoxic groups 108

CORRECTIONS.

Page 8, 14th line from bottom: instead of "Strong," read "Musgrave and Clegg."

Page 12, 4th line from top: instead of "Unstained ability," read "Unstainability."

Page 27: between the 12th and 13th lines from top a line is omitted. It should read: "Other students, especially Pasteur and Koch, soon took up the study of anthrax," etc.; the 13th line is repeated.

Page 57, 14th line from bottom: instead of "varioloid," read "variolata inoculata."

Page 61, 4th line from top: instead of "poisons," read "opsonins."

Page 85, 18th line from bottom: instead of "Part II, Chapter III," read "pages 158-160."

Page 85, 4th line from bottom: instead of "equaled," read "equalled."

Page 107, 13th line from bottom: instead of "flagellæ" read "flagella."

Page 158, footnote: instead of "Chapter III," read "page 264."

Page 181, 3rd line from bottom: instead of "phenomena" read "phenomenon."

Page 206, 4th line from top: instead of "crawfish," read "spider-crab."

Page 217: instead of "opsinogenous," read "opsonigenous."

Page 231, 9th line from bottom: instead of "simulating." read "stimulating."

Page 264: The description of the poison fangs of snakes applies to the chief poisonous snakes of North America, which are "pit vipers"; another class of poisonous snakes, among which are included the cobra, and the coral snake of North America, possess immovable poison fangs.

Page 413, 3rd line from bottom: instead of "toxins of tuberculins," read "toxins or tuberculins."

Page 485, 5th line from bottom: instead of "Nepreu," read "Nepveu."

Page 485, 2d line from bottom, and page 490, 8th line from bottom: instead of "*T. neprevi*" read "*T. nepveui.*"

Page 511, 14th line from bottom: instead of "microscopic," read "ultramicroscopic."

YALE MEDICAL LIBRARY
DEPOSITED
LOANED

YALE MEDICAL LIBRARY
3 9002 01063 8659

locked

Accession no. 31221

Author Ricketts, Howard
Infection, immunity ...

Call no. RC111 R52 1906
HIST

YALE
UNIVERSITY

CUSHING/WHITNEY
MEDICAL LIBRARY