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INFECTION, IMMUNITY AND SERUM THERAPY

In Relation to the Infectious Diseases of Man

By

H. T. RICKETTS, M.D.

LATE ASSISTANT PROFESSOR OF PATHOLOGY, UNIVERSITY OF CHICAGO

SECOND EDITION

Revised and Enlarged by the Author and by Geo. F. Dick, M.D.,
Instructor in Pathology, University of Chicago, with
Preface by Ludvig Hektoen, M.D

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PREFACE TO THE SECOND EDITION

The circumstances that led to the first publication of this book as well as its general scope and character are explained fully by Dr. Ricketts in the preface to the first edition. The book met with such favorable reception that the edition was exhausted while the demand continued active. This indicated that an actual want had been met and so it was determined to publish a new edition, revised and enlarged, and for some time previous to his greatly lamented and untimely death Dr. Ricketts gave freely of his already heavily taxed energies and strength to the work of revision.

Surely a simple word or two in tribute to the memory and achievements of Dr. Ricketts are not out of place at this point. He died in Mexico City, May 3, 1910, at the age of 39, from typhus fever which he was investigating with splendid success when he was taken ill. Thus a noble and inspiring career of large service to humanity and of rich promise came to a sudden and heroic end. During his short but intensely active life as an investigator in the field of infectious diseases Dr. Ricketts made important contributions of permanent value to medical science: he greatly advanced our knowledge of blastomycosis; he solved the most important problems in the cause and transmission of Rocky Mountain spotted fever, and discovered that this disease is conveyed by a tick (*Dermacentor venustus* and *D. modestus*), in which the

infection is hereditary, thereby enlarging our understanding of the part insects may take as carriers of disease; he demonstrated that the typhus fever of the Mexican plateau, *tabardillo*, is carried by the body louse (*Pediculus vestimenti*), and the results of his work on this disease, which he had only just begun, will be of fundamental significance in its prevention and in all future investigation as to its cause and nature. In his death "on the firing line" we lost an investigator of the first rank whose name through these achievements will live in the history of medical science.

When the papers and manuscripts left by Dr. Ricketts came to be examined it was found that while the work of revision and enlargement of this book was very far from complete yet he had carried it so far that it seemed unwise not to attempt to carry it through to completion. Fortunately Dr. George F. Dick was willing to take up the unfinished task, which proved to be a much larger one than was anticipated for the reason that, in addition to completing the revision and adding a considerable amount of new material, it was necessary also to secure proper coordination and balance between the different parts of the book.

The book is now divided into an introduction and three parts instead of two as in the first edition. The Introduction and Part I were prepared by Dr. Ricketts and the chapters on "Sources of Pathogenic Micro-organisms" and on "Special Features of Infection" are new. Part II contains new chapters on "Complement-Deviation," "Opsonins," and "Anaphylaxis," and Part III chapters on "Epidemic Poliomyelitis," "Noma," and "Kala-Azar," all by Dr. Dick. The old chapters in Parts

II and III have been revised by Dr. Dick in the light of recent work and some of them largely or wholly rewritten, notably those on "Syphilis" and "Spotted Fever." As a result the size of the book has been increased nearly 200 pages. It has been the aim throughout to follow the lines laid down by Dr. Ricketts in order to secure an adequate presentation, suitable for physicians in general, of the large subjects of infection and immunity as illustrated by the human infectious diseases in the light of modern knowledge. The present intense and widely spread activity of investigation in these fields, with its many discoveries, is giving us new principles and methods of treatment, curative as well as preventive, and of diagnosis, which require a thorough mastery by the physician in order that they may be used to the best advantage. It is my belief that this book by Dr. Ricketts as revised and enlarged by himself and Dr. Dick will be of real service to the physician who desires a reliable guide to a helpful understanding of the subjects with which it deals. May the use to which it is put show that the labor expended on it shall not have been in vain.

LUDVIG HEKTOEN.

CHICAGO, May 5, 1911.

PREFACE TO THE FIRST EDITION

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 me 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 1905 in *The Journal of the American Medical Association,* 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 356-7. 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 III, attention is called to the explanatory paragraph, page 398.

It will be noted that a 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 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.

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INTRODUCTION

HISTORICAL AND DEVELOPMENTAL.

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 (serum therapy). 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 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 fungous cells and in some instances the as yet unidentified blood and pus cells.

Leeuwenhoek (1632-1723), a Dutch naturalist, with his compound microscope magnifying 1,000 diameters, observed 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 causes 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 scientists, especially Pasteur and Koch, soon took up 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 are due to the action of minute living cells; and, furthermore, that each particular kind of fermentation has its own peculiar microbe as the cause. This was an example of what we term to-day microbic specificity, in marked contrast to views which were then prevalent regarding the variability of micro-organisms.* Pasteur then applied

Microbic Specificity.

* "The following citation from Nägeli illustrates clearly this idea of unlimited variability of microbes: 'In the course of generations the same species assumes alternatingly different morphological and physiological forms which, as years and periods of years pass by, may cause now

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 before it was stored. At the instance of the French government, he then studied certain diseases of silkworms. 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 is smallpox, the virus of which has been attenuated by its passage through the cow, and that consequently when man undergoes vaccination he thereby is 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

souring of milk, now the formation of butyric acid in sauerkraut, now the fermentation of wine, now the decomposition of albuminous matters, now the splitting up of urea, now the red color of starchy food, and give rise now to diphtheria, now to typhoid fever, now to recurrent fever, now to cholera, now to malarial fever.'" (Cited from Hektoen, in Osler's System of Modern Medicine, Vol. I.)

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, Toussaint 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 recovered; 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.

Anthrax.

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 failed, although inoculation experiments proved its presence in this structure. 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 weakening 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 a subject for 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

Exhaustion Theory.

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 suggested 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 in 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 were inaugurated by the findings of Fodor, Nuttall, Nissen, v. Behring and Buchner, which showed that fresh defibrinated blood, and the blood serum of various animals, are 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.

Bactericidal Power.

Toxins and Antitoxins.

Further progress was made through the discoveries 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 antibacterial 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.

Wright in England, Neufeld in Germany, and Hektoen in America more recently have led in a revival of interest in phagocytosis as a factor in natural and acquired immunity, with the result that there can no longer exist any doubt that phagocytosis plays an important rôle in protection against and in recovery from many infections. Thus the opsonins of Wright, and the bacteriotropic substances of Neufeld, have served to bridge any chasm—never a real chasm—which seemed to exist between the so-called humoral and cellular

theories of immunity. The study of opsonins has also served to renew interest in a much-neglected field of specific prevention, namely, bacterial vaccination, one of the most gratifying results of which has been a return to the tuberculin therapy of Koch.

However, with all our resourcefulness, it is possible that our limitations may soon be reached regarding the serum and vaccinal therapy of many infections, and we shall be forced to try other principles of prevention and cure. In connection with this point, the newer chemotherapy, which has been developed in so brilliant a manner by Ehrlich and others in relation to trypanosomiasis, is of the highest interest. It may be hoped that this work represents only the beginning of a new direction of research, which will be of ultimate value in various bacterial as well as protozoan infections.

PART ONE

PRINCIPLES OF INFECTION.

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.

Infectious Disease.

An infectious disease is one which is caused by living organisms which in some way have entered the body, where they multiply and liberate poisonous substances. Accordingly the word has reference to the nature of the cause of the disease. It is from the Latin *inficere,* meaning to place in or into.

Some parasites may live on a host without causing appreciable damage; 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. This is true of organisms which normally inhabit the intestinal tract.

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.

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 and mucous membranes without doing harm, but if certain ones reach the deeper tissues, they may institute pathologic processes (e. g., staphylococci, streptococci, pneumococci, diphtheria bacilli and meningococci). Any organism which is able to cause pathologic tissue changes, to disturb functions, and to set up abnormal symptoms is classed as a pathogenic parasite. The abnormal processes which they institute are our infectious diseases.

Infestation and Infection.

Where certain comparatively large organisms (macroparasites) 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 tapeworms, 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.

Bacteria and Protozoa.

The known pathogenic micro-organisms are grouped among the fungi, the bacteria and the protozoa. Both the bacteria and fungi are vegetable in nature, complexity in form and methods of growth characterizing the latter, whereas the life

history of the former is simpler, and they multiply only by fission (fission fungi). Forms occur which appear to be intermediate between the true fungi and the true bacteria. The protozoa, the lowest forms of animal life, vary greatly in form and in the complexity of their life cycles. The highest protozoa lead an intricate existence in which sexuality and alternation of hosts are sometimes conspicuous features, as in the case of the parasites of malarial fevers, and possibly in that of yellow fever. In some instances the alternation of hosts is purely a facultative property, and not a necessity for the perpetuation of the species, although it is part of the natural cycle; this is the case with some of the trypanosomes, which can be transferred from animal to animal artificially for an indefinite period.

There are certain infections, the causes of which are not known, and in some instances the organisms are considered as ultramicroscopic because they pass through bacteriologic filters. Theoretically they may be either bacteria or protozoa.

Types of Micro-parasites.

The known pathogenic micro-organisms may also be placed in one of the following three groups: 1. Obligate parasites, which are capable of growth only in a living organism (the bacillus of leprosy and the organisms of malaria). 2. Facultative saprophytes, which usually exist as parasites but may multiply on inanimate material under proper conditions. This group includes most of the pathogenic microparasites. 3. Facultative parasites, which are saprophytic organisms living readily on inanimate material, but which may produce disease when they reach suitable tissues in a host

(*Bacillus tetani,* bacillus of malignant edema, *Amœba dysenteriæ*).

Origin and Variations in Organisms.

It is futile to speculate on the ultimate origin of our various micro-organisms. It is sufficient to appreciate that the principles of biology and evolution are broad enough to permit us to assume that some of the species which we now recognize may have arisen through the influences of environment and selection from other more or less closely related species. However, investigations have shown that the essential characters of bacterial species are fixed more or less firmly, suggesting that new species are likely to be developed only through a long course of time, or, if more quickly, through rare chance variations. Koch has suggested that among the trypanosomes found in different diseases some may still be too young in their differentiation to represent fixed species, although this cannot apply to the whole group of trypanosomes.

Many micro-organisms do, indeed, show a great deal of flexibility in their physiology and virulence, with the result that they may approximate species which are usually recognized as being distinct. Thus a strain of the diphtheria bacillus which loses its virulence is similar to a pseudo-diphtheria bacillus, and the cholera vibrio which has become avirulent resembles a number of other vibrios. The plague bacillus, whereas it commonly causes acute death in rats, may undergo such a change in the character of its virulence that it causes a chronic nodular inflammation. Some strains of the tetanus bacillus, which is habitually anaerobic, acquire the power of growing in the presence of atmospheric oxygen. By suitable passage a species of the tubercle bacillus may be

made to resemble very closely in its cultural aspects another species of this organism. On the whole, these are not large variations, and identification can be accomplished by one or more of the biologic reactions, such as the agglutination, bacteriolytic or opsonic tests.

Varieties.

In some instances the conditions are such that different species appear to represent only different grades or types of virulence of the same organism. Those acid-fast bacilli which resemble the bacillus of tuberculosis form the most striking example of this. Bacilli of this character are commonly found in various grasses or clover, which are used as food by cattle. Such organisms have a low grade of virulence, and when injected into animals cause the formation of only a nodule of granulation tissue, sometimes with the presence of giant cells, and the process heals readily. It is possible that certain of these organisms of more than usual virulence, or which may have acquired such virulence by residence in the alimentary tract of the ox, have retained their new pathogenic power as a permanent character, and that bovine tuberculosis originated in this way. It would be none the less logical to assume that the bacillus of human tuberculosis was derived from the bovine type in a similar manner. This can only be a subject for speculation, however.

Bacteriologists frequently are able to place a number of species in a "group," the members of which resemble each other more or less closely, as the colon group, or the dysentery group. The members of a group may vary widely in their pathogenic power, whereas in other instances they produce similar diseases. Thus, Novy has shown

that, although the spirilla which cause the various relapsing fevers are very similar, they can be differentiated by means of immunity reactions, such as the agglutinating or protective powers of immune serums.

A more detailed discussion of this aspect of general bacteriology would carry us too far afield.

"Infectious" and "Contagious."

Confusion sometimes arises concerning the significance of the words infectious and contagious and other words having similar roots. This confusion is due in large part to the fact that they have grown into a usage varying somewhat from that which originally adhered to them, and the dictionaries, even those which are medical in character, have hardly kept pace with the transition.

The significance of *infectious* is indicated in the definition of an infectious disease as given above. The word *contagious,* on the other hand, relates to a method by which some of the infectious diseases are transmitted from an infected person or animal to the healthy, namely, contact, direct or indirect. Not all infections are transmitted by contact, however, hence we may divide them into those which are contagious and those which are not. *Communicable* is often used as synonymous with contagious.

Contagiousness is all the more striking in the case of acute infections which develop rapidly and soon after exposure. On the other hand, it is not so striking in a disease such as pulmonary tuberculosis, which develops slowly and perhaps only after repeated exposures.

The following rather general arrangement of the infectious diseases into groups according to the methods, and at the same time facility, of

transmission is given in order to illustrate the idea and limits of contagiousness.

Facility and Means of Transmission.

1. Those which are characterized by ready transmission through the air. The micro-organisms are discharged into the air from the respiratory passages, or from the skin in desquamative infections, and it is only necessary for a susceptible person to come within the zone of infected air which surrounds the patient in order to acquire the disease. Actual contact with the patient may facilitate transmission in some instances, but is not necessary, and many of them are transmitted by indirect contact, i. e., through the agency of intermediate persons, through food contamination (scarlet fever in milk), or through inanimate substances which have been in contact with the patient. These are all highly contagious "air-borne" diseases, which probably use the respiratory passages as their infection atrium. Diphtheria, scarlet fever, measles, rötheln, smallpox, influenza, tuberculosis, and plague in plague pneumonia are of this type.

Personal Contact.

2. Transmission occurs almost exclusively by personal contact, and usually a special form of contact, never through the air: gonorrhea, syphilis, soft chancre, and perhaps dourine in horses. Syphilis occasionally is transmitted by indirect contact, the free period being a short one. These are contagious diseases.

Indirect Contact.

3. Transmission is chiefly by indirect contact, or through food or water. Direct contact plays a rôle in some instances, and rarely the air may be a means of conveyance. The members of this group are not highly contagious in the sense of transmission directly from one individual to anoth-

er. The micro-organisms are excreted mainly by the feces, and typhoid and paratyphoid by the urine. As a rule they are acquired indirectly, as through a water supply or milk which have been infected from discharges, contamination of the hands from the excreta to food. Examples are typhoid, paratyphoid, cholera and dysentery.

Insects. 4. Transmission by means of insects. Some of these diseases, as malaria, yellow fever, and Rocky Mountain spotted fever, are not contagious at all, but are nevertheless communicable through the medium of the proper insect, mosquitoes or ticks. South African tick fever, ordinary relapsing fever, plague in some instances, trypanosomiasis, and probably typhus are other examples. The steps in transmission are sometimes very complex, and vary a great deal, as will be pointed out later.

Not Transmissible. 5. Transmission from man to man does not take place at all under ordinary conditions: tetanus, hydrophobia, and other wound infections.

It is thus seen that these five divisions constitute a series in which contagiousness finally disappears. The subject of transmission will receive further consideration later.

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 sepa-

rated 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 filariæ, uncinaria).
   - B. Microparasites.
     1. Bacteria (fission fungi: each cell divides into two in proliferating).
     2. Fungi of more complex organization (e. g., aspergillus, oidia).
     3. Protozoa (e. g., *Plasmodium malariæ, Amœba coli*).
     4. Filterable, ultramicroscopic or unknown micro-organisms.

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; the toxins of hay fever).
     2. Bacterial.
        - a. Soluble bacterial toxins (diphtheria and tetanus).
        - b. Intracellular bacterial toxins, which are not secreted by the cells in a soluble form.

In the subjects to be considered we shall deal chiefly with microparasites and the diseases which they cause.

CHAPTER II.

INFECTIOUS ETIOLOGY.

It is evident that the discovery of the specific organism of an infectious disease is of the greatest importance for purposes of serum therapy, vaccination and hygienic prevention. For the demonstration of a virus, it is not in all cases necessary, though desirable, that the organism be cultivated artificially, nor that it be recognized visually. 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 evolved 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).

Also the more recent development of the opsonins and, particularly, of the phenomenon of fixation of complement, promise to be of value in the recognition of specific micro-organisms.

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 trypanosomes, and of Musgrave and Clegg with the *Amœba coli* under symbiotic conditions); second, certain bacteria which may be found constantly in a given disease have not been cultivated artificially (spirillum of recur-

rent fever); third, there are a few diseases which are peculiar to man and accordingly can not be reproduced in experiment animals (leprosy, 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 requirements 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), for the sake of illustration.

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 gonorrhœa*
Epidemic cerebrospinal meningitis	*Diplococcus intracellularis meningitidis* (of Weichselbaum)
Actinomycosis	*Actinomyces bovis et hominis*
Blastomycosis	*Blastomycetes and Oidia*
Malaria	*Plasmodium malariæ*
Syphilis	*Spirochæta pallida*

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 entities because of their constant clinical and anatomical manifestations. Scarlet fever, measles, German measles, chickenpox, smallpox, yellow fever, typhus fever and hydrophobia, 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.

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 is *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. For a long time it was supposed that the so-called hog-cholera bacillus

is the cause of hog cholera. Recent experiments, however, have disclosed that the true virus is ultramicroscopic and filterable, the bacillus being only a more or less constant associate. It is conceivable that in some cases 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. Unstainability 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, yellow fever, hydrophobia, and hog cholera are filterable. This is determined by injecting the filtered culture medium or serum into susceptible animals. The viruses of smallpox, vaccinia, and Rocky Mountain spotted fever are not filterable. Inasmuch as scarlet fever, measles, chicken-pox and typhus fever cannot be produced in animals, the filterability of their viruses is not at present susceptible to determination.

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, together with a constant bacteriology, 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.

Regarding the production of suppuration, 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 in-

vasion of the subcutaneous and connective tissues caused by the streptococcus can often be distinguished clinically from the slower, more circumscribed process caused by the staphylococcus. The conditions 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.

Symbiosis.

In nature pathogenic micro-organisms are often found side by side with saprophytes or with other pathogenic bacteria, and at times their viability is profoundly influenced by their associates. Thus it is found that the bacilli of plague and typhoid and the vibrio of cholera do not live long in the presence of many saprophytic organisms. In some instances this may be due to the exhaustion of the nutrient material by the more rapidly growing saprophytes, whereas in others it may be referable to an antagonistic action of one organism on the other. On the other hand, the relationship may be a favorable one. The existence of anaërobic organisms in nature, such as the tetanus bacillus or the bacillus of malignant edema, may be favored by a luxuriant growth of aërobic organisms in their immediate vicinity.

This relationship is investigated experimentally either by growing two organisms in mixed cultures, noting subsequently whether one has outgrown the other, or one may be grown on a medium which has previously been utilized by the other. The predominance of one or the other may depend on the nature of the culture medium. This principle is utilized in obtaining pure cultures of the cholera vibrio from dejecta by the use of a strong alkaline medium which favors the growth of the cholera vibrio but inhibits that of the other intestinal bacteria. Similarly the diphtheria bacillus grows well on Loeffler's blood serum, whereas the other organisms commonly found in the throat do not.

Garré distinguished a one-sided and a mutual antagonism between bacteria, but the former seems to be the more common. Examples of favorable symbiosis on suitable culture media are the following: Streptococcus and cholera vibrio; anthrax and pyocyaneus bacilli (Turró); diphtheria bacillus and the streptococcus (Hilbert). In plate cultures it has been found that the influenza bacillus produces unusually large colonies when they lie adjacent to colonies of the staphylococcus (Grassberger), and that it grows vigorously on agar which contains killed bodies of the gonococcus or diphtheria bacillus (Cantani). It has been noted that the diphtheria bacillus is stimulated to a greater production of toxin by the presence of streptococci.

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 possible that they inaugurate 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 and vaccinal therapy. Experience regarding the former is limited practically to diphtheria, and there is no lack of evidence to show that the disease when complicated by severe streptococcus infection sometimes cannot be controlled by antitoxin treatment; and in vaccinal therapy (injection of micro-organisms or their products) it is emphasized from all sides that in the presence of mixed infection it is advisable to inject preparations of the secondary as well as the primary organisms concerned.

CHAPTER III.

INFECTION ATRIA AND THE EXCRETION OF MICRO-ORGANISMS.

Infection Atria.

The infection atrium is the primary point of invasion by micro-organisms, or the point or tissue or surface through which they reach internal structures.

In general, micro-organisms may enter the body through any of its surfaces, except, of course, the serous coverings. Anatomical structure, however, renders the skin, and other surfaces which are clothed with pavement epithelium, quite resistant to penetration, in the absence of wounds. Leaving wound infection out of consideration the mucous surfaces afford the most frequent points of entrance.

Preferred or Specific Atria.

Certain micro-organisms appear to have a predilection for particular tissues, preferring one point of entrance or primary involvement above all others. Thus, in so far as we know, the typhoid bacillus and the cholera vibrio always produce their primary infection in the intestines, although a hematogenous typhoid is sometimes spoken of, the true atrium escaping detection. The diphtheria bacillus habitually makes its attack in the upper respiratory passages. It is probable that measles and scarlet fever utilize the respiratory tract for the point of invasion, although this cannot be determined positively at present. As will be described later, many microbes have a predilec-

tion for certain tissues after they reach the interior of the body.

Sometimes it would appear that a particular surface is predilected only because it is the area which is most commonly exposed to infection. Thus, syphilis is usually a venereal disease, although on proper exposure chancres occur readily on the lip, the mucous membranes of the mouth, tonsils, or through wounds in the skin. Hence the predilection of the primary sore for the genitals is only apparent. Also the diphtheria bacillus occasionally is inoculated into wounds on cutaneous surfaces and in the vagina. Some mucous surfaces are protected against microbic invasion by the character of their secretions, as in the cases of the stomach and vagina (See under "Natural Immunity").

Cryptogenetic Infections. In the so-called cryptogenetic infections the atrium escapes detection. Certain micro-organisms may enter the body without causing a discoverable reaction at the point of entrance (plague). Experimentally it has been shown that tubercle bacilli readily pass through the intestinal wall into the mesenteric lymph glands without causing lesions of the intestines. Leucocytes may carry organisms through the intact surface of the intestines into the deeper tissues, and possibly the same process occurs in the lungs, particularly in relation to the tubercle bacillus (!).

Skin. Infection through the skin commonly takes place through wounds (tetanus, glanders, malignant edema and purulent infections), although the wound may be so small as not to be discoverable (bubonic plague). "Insect-borne" diseases are inoculated through the skin by the bites of the

insects. In the case of plague the bacilli may be deposited on the skin in the feces of the flea and subsequently inoculated by means of rubbing or scratching. The guinea-pig may be infected with plague by rubbing a culture on the shaven skin. Minute wounds probably exist. Staphylococci may reach the hair follicles as a consequence of rubbing and cause furunculosis after penetrating the soft epithelium of the follicle.

Eye.

The conjunctiva has rather high resistance for some micro-organisms, as the anthrax bacillus, and it harbors staphylococci continuously. It may be invaded, however, by the gonococcus (especially in children), pneumococcus, streptococcus, staphylococcus, diphtheria bacillus, Morax-Axenfeld bacillus, and probably the meningococcus. The plague bacillus will cause generalized infection through the conjunctiva in rats, and it is reported that glanders and hydrophobia (Conté, Galtier) may also gain entrance through the conjunctiva.

Nasal Cavities.

The nasal passages become infected with the organisms causing coryza, with the organisms of diphtheria, influenza, glanders, leprosy and with the pyogenic cocci. Some of these may extend to the adjacent cavities, antrum of Highmore, frontal sinuses, and the middle ear through the Eustachian tube. On account of the proximity of the nasal passages to the brain, and the lymphatic communications, it is probable that meningitis (pneumococcic and epidemic cerebrospinal) often arises by extension of the organisms from the nose to the meninges through the ethmoid.

Mouth.

Actinomycosis, syphilis, occasionally tuberculosis (tongue), noma, thrush, and in children gonorrhea may find primary location in the mouth.

Tonsils. Streptococci, pneumococci and diphtheria readily attack the tonsils, and it is probable that the tubercle bacillus often enters through them, with or without causing local infection. Similarly it is believed by many that organisms causing septicemia (particularly the streptococcus), acute articular rheumatism, and osteomyelitis may enter through the tonsils, and this may also be the case in scarlet fever.

Lungs. The bronchi become infected by the various organisms causing bronchitis (streptococcus, pneumococcus, influenza bacillus, etc.), and, either through surface or lymphatic extension, or by deep inspiration, these and many other organisms, as the tubercle and plague bacilli, and the actinomyces, reach the deeper recesses. Some of the exanthemata, as measles and smallpox, may find entrance through the pulmonary tissue. As explained later, "dust infection" and "droplet infection" are of great importance in pulmonary involvement, particularly in relation to tuberculosis. "Primary" tuberculosis of the peribronchial lymph glands indicates that some micro-organisms may traverse the bronchi without involving them.

Stomach and Intestines. The stomach is comparatively free from infections. The intestines provide an atrium for typhoid, cholera, dysentery (bacillary and amebic), tuberculosis, plague, anthrax, in children for the streptococcus, *Bacillus pyocyaneus* and others; in animals, for anthrax, plague, swine plague, mouse typhus, chicken cholera, hemorrhagic septicemias, intestinal diphtheria of rabbits, and others.

The extent to which micro-organisms are carried from the intestines into the body in a state of health is greatly disputed, but frequent existence

of primary tuberculosis of the mesenteric lymph glands indicates that it probably occurs.

Rectum and Genito-Urinary Tract.

Rectum.—Gonorrhea, septic infection of hemorrhoidal veins.

Urethra.—Gonorrhea, syphilis; "simple" urethritis due to other causes.

Bladder and Ureters.—Infection usually secondary, by extension or from the blood stream.

External Genitals.—Hard and soft chancres. Diphtheria in girls.

Female Genital Tract.—Gonorrhea, especially in the cervix, which is covered by soft cuboidal epithelium. The vagina is quite resistant owing to its covering of pavement epithelium and the bactericidal character of its secretion. Virulent pyogenic organisms sometimes are found in the normal vagina and they may occasionally be responsible for puerperal infections.

The above refers to primary invasion. It is well known that many of these surfaces and the organs which they cover are frequently involved subsequent to initial infection at some other point. This is secondary, or, better, metastatic infection.

Excretion of Micro-organisms.

In the transmission of an infection from person to person, without the intervention of an intermediate host (insects), the process naturally presupposes that the micro-organisms are excreted or discharged from the body of the patient through one channel or another. Regarding the likelihood of transmission, in the event of excretion, this will depend on the character of the organism, its viability under the conditions of excretion, the infection atrium which it demands, and whether or

not it is discharged in such manner that it is readily disseminated. Some of these points are considered in following chapters.

Relation of Excretion to Site of Involvement.

As a general principle it may be stated that when the surfaces of the body are the seat of infection the micro-organisms are discharged into the outer world more or less easily. This applies not only to the cutaneous surface, but also to the mucous surfaces, as the lungs, alimentary tract, gall-bladder, and urinary bladder, and also to discharging sinuses and abscesses which rupture through a cutaneous or mucous surface. The surfaces may be involved either primarily, as in tuberculosis or blastomycosis of the skin, or as a part of a generalized infection, as in the case of some of the eruptive diseases. Thus from the respiratory passages the microbes of pneumonia, tuberculosis, diphtheria, tonsillitis caused by other micro-organisms, plague pneumonia, influenza, whooping-cough, epidemic cerebrospinal meningitis and probably measles, scarlet fever and smallpox, are discharged into the surrounding air. From the intestinal tract the micro-organisms of tuberculosis, typhoid fever, cholera and of other less important diseases reach the outer world. From the genito-urinary tract, those of gonorrhea, syphilis, tuberculosis, typhoid and paratyphoid fevers; from the skin the microbes of ulcerative processes, the contagious dermatoses, trichophytosis, favus, etc., and probably some of the contagious exanthemata (scarlet fever, measles, etc.).

Metastatic Infections.

Of great importance is the fact that some infections which are primarily systemic, or become so during the course of infection, commonly involve some excreting organ secondarily, thus rendering

possible, or increasing, the discharge of the organisms. In a large percentage of the cases of typhoid fever the kidneys become the seat of numerous foci of metastatic infection, resulting in the elimination of large quantities of living, virulent typhoid bacilli in the urine. A similar event happens in paratyphoid fever, and in systemic tuberculosis, and Koch has even suggested that sleeping sickness may be acquired by coitus. "Milk sickness," which apparently is acquired through the gastro-intestinal tract, is transmitted through the milk (Jordan and Harris), and the same is true of Malta fever. The virus of hydrophobia is excreted through the salivary glands. Experimentally, pneumococci and anthrax bacilli, when injected into the circulation, have been recovered from the intestinal tract. This was also done with the vibrio of cholera in guinea-pigs (Kolle and Issaeff).

"Carriers."

These statements are made with reference to the discharge of micro-organisms during actual disease. Investigations have shown, however, that infections on a large scale, reaching even epidemic proportions, are frequently derived from those who apparently are in a state of good health. Usually, but not always, this concerns individuals who have suffered from the infection at some previous time, and they are known as "bacillus carriers," or simply "carriers."

The vibrio of cholera may be excreted in the stools for forty-eight days after the recovery of the patient (Kolle). Virulent diphtheria bacilli may be discharged from the mouth and nose for months after recovery from the disease, and in the case of *rhinitis fibrinosa chronica* this may persist

for years. "Latent" gonorrhea is frequently infective. The urine after recovery from typhoid fever may contain the bacilli for months (Petruschky and others), and the recent study of typhoid carriers has shown that they may discharge bacilli in the stools for many years (twenty or more). In some instances it has been supposed that the gall-bladder is in a state of chronic infection with the bacilli and serves as a reservoir for continuous flooding of the intestines. The value of the remedial measure suggested—i. e.., the extirpation of the gall-bladder—has not yet been demonstrated. We cannot leave out of mind the possibility that the typhoid bacillus, in some instances, may become habituated to the intestinal environment and multiply there; in such cases it would not be necessary to assume another source of replenishment. The dissemination of microorganisms by insects which have fed on infected blood is apart from excretion and is considered under the subject of "Insect Transmission" Chapter VI.).

CHAPTER IV.

SOURCES OF PATHOGENIC MICRO-ORGANISMS.

(1) *Earth, Etc.;* (2) *Food Substances;* (3) *Animals;* (4) *Body Surfaces of the Individual.*

Micro-organisms which produce disease in man may be derived (1) from the earth, other inanimate material, or from vegetable growth; (2) from water, milk or other food substances; (3) from animals, directly or indirectly; (4) from the body surfaces of the individual himself; (5) from other human beings, and (6) from insects.

Earth.

1. The extent to which the superficial earth is contaminated with micro-organisms depends on various conditions, particularly the presence of dead organic matter, moisture, temperature, the degree of exposure to light and sunlight, the chemical composition of the soil, and the admixture of animal excretions. Some of them inhabit the soil naturally and are comparatively harmless saprophytes, and may even be of great value in the regeneration of soils. Others, of pathogenic character, seem to occur naturally in the earth or on vegetation, where they multiply readily (the pyogenic cocci, actinomyces). On the other hand, a large number of pathogenic organisms reach inanimate nature only as they are deposited with the excretions of man (those of typhoid, paratyphoid, dysentery, cholera, plague, tuberculosis, etc.), or of animals (tetanus bacilli). Many, as the organisms of typhoid, cholera and plague, probably do not proliferate at all in the earth, although some (tet-

anus bacillus and that of malignant edema) retain life and virulence for a long time. It is still uncertain whether the latter proliferate in this situation, or whether they persist merely through the agency of their resistant spores. Inasmuch as they are anaërobic in character, their life may in some instances be prolonged through symbiotic aërobic organisms, which surround them and create for them an atmosphere which is poor in oxygen. The presence of many saprophytes is unfavorable to the life of certain micro-organisms in the soil (typhoid, plague). In so far as is known none of the protozoa which are pathogenic for man occur in the soil naturally.

Water, and Other Food Substances.

2. Water, milk and other food substances rarely harbor important pathogenic organisms under natural conditions. On the other hand, they play a very important part in carrying such microbes from man to man, and in some instances from animals to man, as explained in succeeding chapters. Stagnant water frequently sets up acute enteritis, which may in some instances be due to its chemical constituents and in others to saprophytic organisms.

Solid foods, as fruits and vegetables, may occasionally harbor bacteria, particularly when in a state of decay, which have moderate pathogenic powers for the intestinal tract; or, by providing suitable alimentation or otherwise modifying the contents or resistance of the alimentary tract, may render organisms virulent which otherwise would be harmless.

Meats, healthy in the first instance, are subject to invasions by micro-organisms from the intestinal tract, after the death of the animals (fowls,

fish, oysters), or they may be contaminated by unclean and improper preservation later. The symptoms caused by their ingestion are actual infections in some instances (as with *Bacillus enteritidis*), whereas in others the condition may be one of intoxication by the products of saprophytic activity (*Bacillus botulinus*).

Some vegetables contain highly poisonous alkaloids and toxins, which rarely find a place in disease. Some of them have been of great value in the experimental study of toxins and antitoxins. (See table at close of Chapter I.)

Animals. 3. Animals are sometimes subject to infection by microbes which are also pathogenic for man, transmission taking place through the consumption of diseased meat or milk, or through direct or indirect contact, through wounds, or by the bites of insects.

Milk. In bovine tuberculosis the udder is frequently involved, and in such cases large quantities of the bacilli are excreted by the milk. Although the virulence of the bovine bacillus for man may not be so great as that of human tuberculosis, it is now well established that it often infects man, perhaps children more frequently than adults. The cow's udder is occasionally involved in infections with streptococci, which, being excreted in the milk, are capable of causing severe enteritis when the milk is ingested.

The micro-organism of milk sickness is transferred in a similar way, and only recently it has been shown clearly that man (in Malta) becomes infected with Malta fever by the consumption of the milk of goats. A very large percentage of these

animals was found suffering from the disease, and, since the use of goat's milk has been prohibited, the incidence of the disease in man has undergone an astonishing decrease. It seems to have been demonstrated that both horses and cattle occasionally suffer from generalized infections with the paratyphoid bacillus, and with *Bacillus enteritidis*, and epidemic infections with the former have been traced to the consumption of diseased meats. Botulism and trichiniasis are derived in the same way. In some instances infection of the meat probably takes place during or subsequent to slaughtering.

By Contact. Anthrax, glanders and actinomycosis are conveyed to man from animals by contact, the first two being contagious; and hydrophobia by the bites of rabid animals—i. e., by wound infection.

By Insects. In a few instances infections are transmitted from an animal to man by means of insects. Thus, the conveyance of plague from the rat by the flea is one of the means by which man contracts this disease. The tsetse fly of South Africa, which inoculates man with the trypanosome of sleeping sickness, seems to derive its infection from wild animals in some instances, although human patients are also an important source of infection for fresh flies. The virus of Rocky Mountain spotted fever probably passes at least a part of its existence in the body of one or more species of small wild animals, and this step seems necessary for the maintenance of virulence. In connection with malaria, relapsing fever, South African tick fever and the piroplasmoses a third host seems to play no rôle, or, at any rate, not a necessary rôle.

Snake venoms, and the poisons of spiders, bees, and various insects, are toxins of animal origin which have pathologic and scientific importance.

4. "Endogenous" infection or "autoinfection." By this term we mean infection of an individual by micro-organisms which reside naturally on some surface of the body. Such organisms produce infection only when some other factor, particularly traumatism, comes into operation. **"Auto-Infection."**

Hair follicles frequently contain staphylococci and when occlusion occurs the organisms may produce a pustule or a furuncle. An injury of the conjunctiva may result in infection by staphylococci or pneumococci, which are present normally, and many wound infections are due to organisms which pre-exist on the surface.

Probably a factor of great importance for the invasion of such organisms is a condition of lowered resistance on the part of the tissues. We know, for example, that virulent streptococci and pneumococci are frequently found in the pharynx and on the tonsils in apparent health. Exposure to cold in some instances may be the means of lowering the resistance of the surfaces (inhibition of the antibacterial forces) so that the organisms become more numerous and penetrate the surface. Observations suggest also that the presence of a serous exudate, such as exists even in a transient inflammation, may cause an increase in the virulence of the organisms which are bathed by it. Similar forces may play a rôle in the bronchitis and pneumonia which follow exposure.

There seems to be comparatively little danger of "autoinfection" from pathogenic bacteria which exist normally in the intestinal tract, except in

the case of traumatism, as in incarcerated hernia, or in severe constipation, or when the normal resistance of the intestines has been much disturbed by improper food.

Relapses. Sometimes an attack of a disease represents a recrudescence or reinfection by organisms which have persisted at some point following a previous attack. Relapses of typhoid fever and recurrences of facial erysipelas, and frequently the flaring up of an old (latent) gonorrhea, illustrate this. Acute miliary tuberculosis, or tuberculous meningitis, may follow the escape of bacilli into the circulation from an unsuspected focus in a peribronchial lymph gland. This manifestly is not autoinfection or endogenous infection, since, even in the old latent foci, the infection dates back to a prior invasion.

CHAPTER V.

SOURCES OF PATHOGENIC MICRO-ORGANISMS

(Continued.)

(5) *From Man to Man.*

As indicated in Chapter I. there are all degrees in the facility with which infectious diseases are transmitted from one person to another, varying from that in which it is only necessary to breathe the air surrounding a patient (scarlet fever, measles, influenza, etc.), to that in which transmission never takes place under ordinary circumstances (tetanus, hydrophobia).

Relation of Atrium to Method of Excretion.

In some instances a logical relationship exists between the facility of transmission, on the one hand, and the form of excretion of the micro-organisms and their preferred infection atrium, on the other. Thus in many diseases in which the micro-organisms are excreted from the respiratory passages the latter are also used as the infection atrium (influenza, measles, smallpox, etc.). In others, in which they are excreted mainly by the intestines, the infection atrium is the intestines (typhoid, cholera, dysentery). Even if it were possible for the organisms of typhoid and cholera to gain entrance through the lungs (and indeed it may be possible in typhoid at least), the fact of their excretion mainly by the stools, and by the urine in typhoid, would render primary pulmonary involvement difficult. They reach the intestines more readily through contaminated food, water,

etc., than they could reach the lungs through infected dust or droplets. Similarly, if the virus of scarlet fever, which is excreted from the lungs and skin, could cause infection only through cutaneous wounds, rather than through the air passages, there is reason to believe it would not hold its present position as a very contagious disease. Among the communicable diseases we have to recognize that each has its own mechanism for habitual transmission, although the habitual mechanism may be departed from on many occasions.

Mediums of Conveyance.

As would be supposed, the diseases which are most readily acquired, the most contagious, and the most prevalent are those in which the microorganisms are excreted into the air from the respiratory passages, and in which also the respiratory passages are the preferred infection atrium. The medium of conveyance—i. e., the air—is used alike by all individuals. Some other diseases, much less contagious than those mentioned, may prevail in extensive epidemic form, often exceeding scarlet fever, measles, etc., in the percentage of incidence, as in the cases of cholera and typhoid fever. This is commonly due to an infected water supply, and the distribution of the disease corresponds in large degree to that of the contaminated water. In other words, the epidemic is coextensive with the use of the conveying medium.

Similarly, the prevalence of gonorrhea and syphilis depends on the extent of promiscuous sexual intercourse; and, of malaria and yellow fever, on the number of individuals who become bitten by the infected Anopheles or Stegomyia mosquitoes.

Thus the central factor in the communication of infection is suitable contact with the agency

of conveyance, and where such contact can be realized with difficulty communication from person to person rarely occurs (e. g tetanus and hydrophobia, in which wound inoculation is required).

Air as Conveying Medium.

It is probable that the air, in the absence of atmospheric currents, would soon become sterile, by virtue of the effect of gravity in carrying the microbes to the earth, and the germicidal action of sunlight and diffuse light. Of course, these conditions do not prevail, or are not effective, and the air stands as one of the important agencies by which virulent micro-organisms reach the individual, either from other individuals and animals or from inanimate nature.

For a long time it was supposed that conveyance through the air takes place chiefly or entirely through the medium of fine particles of dust which are laden with micro-organisms ("dust infection"). It was only through fundamental work by Flugge (1897) and others that it was shown how minute droplets of saliva or mucus from the lungs or nasal passages are fully as important as dust, and perhaps more important, for the transfer of infection from one individual to another. This is "droplet infection."

"Dust Infection."

The fields occupied by dust infection and droplet infection do not coincide exactly. Only those diseases can be concerned in droplet infection in which infected droplets are discharged from the body—i. e., chiefly from the upper respiratory passages. Since the infective droplets of saliva, serum, mucus and pus may become desiccated and pulverized, the field of dust infection, theoretically, includes not only those diseases, but also many others. Thus, dust infected with the pyogenic or-

ganisms, or with tetanus bacilli, may be derived from ordinary earth or earth contaminated with the dried excretions of animals. And in other cases dried and pulverized urine or feces (typhoid. cholera), or desiccated discharges from diseased surfaces, from sinus and abscesses (tuberculosis, erysipelas), may give rise to infected dust. The skin may be an important source of infected dust in the desquamative diseases (scarlet fever, measles, smallpox), in that individual horny cells or groups of cells laden with organisms may float in the air with no great velocity in the movement of the latter.

The importance of dust infection is curtailed, however, because of the slight resistance which many microbes show against desiccation and the germicidal action of light. Gotschlich classifies micro-organisms regarding the likelihood of their participating in dust nfection as follows:

Classes of Organisms.

(a) Organisms which are not capable of living in dust as it is dried in the air, and hence never (*rarely* might be better) can be disseminated through dried particles (cholera, plague, gonorrhea, influenza).

(b) Organisms which are capable of being carried as dust for considerable distances by such weak currents of air as ordinarily exist in dwellings, and which, when once suspended in the air, remain alive for a long time, and easily lead to dust infection (pus cocci, pyocyaneus, meningococcus, anthrax spores, tubercle bacilli and tetanus bacilli).

(c) Organisms which, indeed, are resistant to desiccation, but which are disseminated as dust only through stronger air currents, such as occur

exceptionally in dwellings (typhoid and less often diphtheria).

Conditions in Dwellings.

This leaves out of consideration the unknown organisms of the exanthemata, referred to above. The conditions as they ordinarily exist in dwellings are taken as a standard, and the weight and size of the particles, and the velocity of the air in an upward direction, as well as the viability of the different organisms in a dried condition, are the essential factors which govern the likelihood of dust infection. "Naturally, only those infected particles of dried dust which may be carried upward for a considerable distance by a very low velocity of the air are able to remain suspended for some time in the atmosphere of a room and, consequently, exist as a protracted danger of air infection. M. Neisser considers as the limit that degree of "Verstaubbarkeit"* in which the infected particles may be carried to a height of 80 cm. by an air velocity (upward) of 1 cm. (per second)." (Quoted from Gotschlich in Kolle & Wassermann's Handbuch der Pathogenen Mikroorganisms, Vol. 1, p. 168). After the dust in a room is once thoroughly stirred up, from one to eight hours are required for it to settle completely under the most favorable conditions (Stern, Flügge, cited by Gotschlich). Dust infection may play a part in public buildings and conveyances where the currents of air are stronger than they ordinarily are in dwellings. Even in dwellings, however, the conditions are by no means uniform. More violent currents are excited by a breeze through an open window, by the movements of persons, and the chances of infection are increased

* Pulverization.

as a consequence of "dusting." Naturally the longer the suspension within the period of viability of the organisms the greater the chance of conveyance.

Droplet Infection.

Droplets of saliva, etc., are discharged into the air through coughing, sneezing, laughter, and even forcible speaking. This does not occur with ordinary respiration. In coughing droplets may be carried as far as thirty feet from the individual, and they may be carried much farther by extraneous currents of air. Such droplets may remain suspended in the air for approximately an hour, but the period of their suspension depends on their size, the weight of the organisms they carry and the degree of moisture in the air. In a drier air they become desiccated more quickly and after desiccation fall more rapidly.

Laschtschenko, and also Heymann, investigated the dissemination of tubercle bacilli by coughing in pulmonary tuberculosis. In 40 per cent. of the cases guinea-pigs which were placed even at considerable distances from the patients contracted tuberculosis of the lungs or bronchial lymph glands. The bacilli have been demonstrated microscopically in such droplets by numerous observers. Leprosy bacilli are carried similarly (Schäffer).

On account of their content in mucin the droplets adhere closely to solid surfaces, where they soon dry and become harmless unless they are dislodged by some violence.

When contained in such dry droplets the tubercle bacillus lives for about three days in the light and eighteen days in the dark. Most other organisms, except the spore-bearing and those caus-

ing some of the contagious exanthemata, die more quickly.

Conditions.

The conditions for droplet infection, then, are, in the main, as follows: *First,* the micro-organisms must be discharged into the air, in viable and virulent condition, and in sufficient quantity, from the respiratory passages of the patient. *Second,* they must be able to retain life and virulence for a greater or less period of time, after being liberated in this way. *Third,* they must be able to use some portion of the respiratory tract, or the gastro-intestinal tract indirectly from the respiratory tract, as an infection atrium. *Fourth,* the advent of a susceptible person within the zone of infected atmosphere which surrounds the patient during the period of viability and virulence of the excreted organisms.

In general the same principles also apply to dust infection so far as it concerns the ordinary "air borne" diseases.

Relative Importance.

The question is commonly raised as to which is the more important, or more prevalent, dust infection or droplet infection. Although it is the tendency at present to assign a minor rôle to dust infection, it unquestionably is of importance in certain diseases, particularly in tuberculosis. The situation may be conceived to be as follows: In relation to diseases caused by micro-organisms which have little resistance to desiccation and light (e. g.., plague, influenza), droplet infection, implying proximity to the patient, is more likely to occur than dust infection (as in occupying a room vacated by a patient a longer or shorter period previously). Concerning those caused by organisms which have greater resistance to desicca-

tion and light, as in tuberculosis, the importance of dust infection approximates, though it may not equal, that of droplet infection.

Water as Conveying Medium.

Just as the air is the principal medium of conveyance for the group of diseases discussed above, so contaminated water plays an important, but not the sole, part in the transmission of another group. Typhoid and paratyphoid fever, cholera and bacillary dysentery are the chief representatives.

Epidemics which arise in this way are frequently spoken of as "water-borne" epidemics. Sometimes typhoid and cholera are called "water-borne" diseases, but epidemics are so often instituted and maintained by various kinds of indirect contact that the appellation is one-sided.

Conditions.

Three essential conditions are required in order that a disease may be more or less habitually transmitted through water: First, the discharge of the organisms from the body of the patient in such form that they may reach a water supply. Second, the ability of the organisms either to live for a moderate length of time in the water, or to proliferate in it, without losing virulence. Third, the utilization of the gastro-intestinal tract, the upper respiratory tract, or the lungs indirectly from the latter, as an atrium of infection.

The first condition is readily realized in the diseases mentioned, inasmuch as the micro-organisms are discharged in large numbers with the feces, and also, in the case of typhoid and paratyphoid, with the urine.

Sources of Contamination.

All types of water supplies may be contaminated by these excretions. When the water of a community is taken from a stream the latter may be infected by the sewage of another community

higher up the stream, or by the discharges of even a single patient. The throwing of typhoid discharges on the bank of a stream has resulted in severe epidemics. Reservoirs may be infected similarly. In some instances a city which derives its water from an inland lake also empties its sewers into the same body of water. Even when the sewage outlet is quite remote from the water intake, surface currents, as caused by the wind, may carry water, and hence infection, from the former to the latter. In harbors the water may become infected from the sewage of a ship which carries a case of cholera. Streams have been contaminated by washing in them the soiled linen of patients. When excretions are thrown on the ground the micro-organisms have been carried into wells by surface water from which small epidemics have arisen.

Viability in Water.

The very occurrence of water-borne epidemics indicates that the micro-organisms concerned live for a longer or shorter period of time in ordinary waters. Various factors influence their longevity in water, and their persistence at the point of first contamination. Purer waters are not so favorable for the life of the organisms of typhoid and cholera as those which contain a certain amount of organic matter and salts. On the other hand, an excess of organic matter when accompanied by many saprophytic organisms also shortens the life of these bacteria, particularly when in stagnant water; and this principle is utilized for purification of sewage in those systems which involve the use of sewage tanks. A rapidly flowing stream naturally results in purification more quickly than one which is sluggish.

Data regarding the longevity of these organisms in water and milk are given in the sections on cholera and typhoid.

"Water-Borne" and "Contact" Epidemics.

"Water-borne" epidemics are characteristic in this, that very many individuals are stricken suddenly and simultaneously and the outbreak is, at first, limited to those who are supplied by the infected water. "Contact" epidemics, on the other hand, progress slowly and irregularly, although they may finally reach large proportions. Naturally an epidemic begun by contaminated water may be maintained by contact, and continuance by contact again offers opportunity for the fresh infection of water, milk and food. The viability of micro-organisms in the excreta, after the discharge of the latter, is important, both from the standpoint of water infection and that which occurs by indirect contact. Uffelmann determined that typhoid bacilli may live in the dejecta for many months, and, at least under some conditions, the cholera vibrios in the feces is viable for two or three weeks (Lubarsch).

Conveyance by Food.

As stated previously, diseases which are peculiar to man may be distributed by milk which has been contaminated by convalescents (as in scarlet fever) or by "carriers," or by some others indirectly from these, or by infected water used in washing containers. Epidemics caused by infected milk are, in miniature, similar to those arising from a contaminated water supply, their distribution coinciding with the area of consumption of the milk.

Other food substances act as carrying agents only when they become infected accidentally, as by flies, washing in contaminated water, or by convalescents and "carriers."

Contact.

Transmission by direct and indirect contact are somewhat in contrast. In the former actual personal contact takes place between the sick and healthy, as previously stated, and as illustrated by gonorrhea and syphilis.* Probably many of the infections which are conveyed through the air may also be acquired by direct contact. However, a distinction in this case has no significance, since actual contact without exposure to air infection could hardly occur.

Indirect contact, on the other hand, implies transmission through the agency of an intermediate person or object. Speaking strictly, conveyance from person to person, through the air, water, or even by insects, comes within the domain of indirect contact, yet their methods are so specialized, so obtrusive and so constantly utilized by certain groups of micro-organisms that they deserve the separate consideration usually given them. The tendency is a correct one to withdraw from the domain of indirect contact any method of transmission which can be spoken of more concretely.

One could not hope to mention all of the possible channels through which an infection may be carried indirectly. There are great variations in details. "Carrying a disease in one's clothing" from place to place; the use of the toys of a diphtheritic child; washing or handling the linen of a cholera, typhoid or dysentery patient; occupying a room formerly used by a patient having scarlet fever or tuberculosis; the occasional transfer of syphilis by the drinking-cup or the dentist's for-

* Exceptions occur in relation to syphilis, as in the occasional transfer by drinking cups and instruments; hereditary syphilis may be considered as a special case.

ceps; the former occurrence of contagious hospital gangrene through contamination of dressings and of other infections from patient to patient through unclean instruments or hands; these are examples of conveyance by indirect contact.

As some of them show, diseases which are habitually transmitted through the air (scarlet fever) or by water (typhoid, cholera) may also be transferred by indirect contact.

Hereditary Transmission.

In the strict zoological sense no form of transmission of an infection from parent to offspring can be viewed as truly hereditary, since inheritance concerns only properties which are inherent in the germ cells and their chromatin. Micro-organisms are foreign and their introduction in the germ cells from the parent can only be considered as accidental. It is, then, only for the sake of convenience and for lack of a more exact term that the inheritance of infections is spoken of. This distinction has been strongly emphasized by Hansen (Virch. Arch., Vol. 120) and by Lubarsch (Ibid., Vol. 124). Some of the metabolic diseases and functional derangements, on the other hand, may be truly inherited, or, perhaps better, a tendency or predisposition to them may be inherited. Also, it is very probable that susceptibility, or, on the other hand, resistance to some particular infection, may be inherited, thus accounting for the frequent or rare occurrence of some infections in a given family.

Germ Cell Infection.

When disease of the offspring can be referred to a primary invasion of the germ cells (ovum or sperm cells) by micro-organisms it is said to have originated by "germ cell infection." It is not definitely known that this type of hereditary trans-

mission takes place in man, and the likelihood of its occurrence could be proved only by finding the micro-organisms actually within the ovum of sperm cell.

Definite examples of this type of transmission are found in insect life, particularly among ticks. The piroplasmas of Texas fever and of Rhodesian fever of cattle, the spirillum of South African tick fever of man, and the virus of Rocky Mountain spotted fever are all transmitted to the larvæ of the next generation through infection of the ova of the corresponding ticks.

Since it does occur in other forms of life, it would not be surprising if it also occurs in man. In that form of inherited syphilis in which the child derives the infection from the father, the mother apparently remaining uninfected, the virus may have been introduced into the ovum by means of an infected sperm cell. Even in this case, however, it is virtually impossible to rule out the existence of a latent infection of the mother. And if such infection does exist, the spirochetes may have reached the embryo by way of the placenta, instead of through the ovum. Possibly the recently discovered test for syphilis (fixation of complement) will throw some light on this phase of inherited syphilis, since it renders possible the diagnosis of the disease in the mother regardless of positive clinical manifestations.

It is equally, or perhaps more, uncertain as to whether tuberculosis is ever inherited through infection of the germ cells. Tubercle bacilli have been found in the testicular secretion in both man and animals. In such cases the tuberculosis is of an advanced type, resulting in early death. Even

if some of the bacilli were actually within the sperm cells and capable of introduction into the ovum through them, the great preponderance of the spermatozoa over the bacilli renders infection of the ovum improbable. In one such case Gärtner estimated the numerical relation of the bacilli to the spermatozoa as one to 22.7 million, hence the impregnating cell would very likely be an uninfected one.

It has sometimes been assumed that leprosy may be transmitted in this way, but there is no strong evidence in support of it.

Placental Transmission.

Infection of the embryo, from the mother, by way of the placenta, has been demonstrated experimentally, and encountered clinically, unmistakably. Opinions differ as to whether actual involvement —i. e., infection or defects—of the placenta is prerequisite to the passage of micro-organisms from the mother to the embryo.

M. Wolff, on the basis of experimental work with the anthrax bacillus, concluded that the uninjured placenta is an effective barrier against such a transfer. It was supposed, however, that injuries of the placenta which resulted in bleeding, thus establishing a temporary connection between the circulations of the mother and fetus, as well as other lesions metastatic in character, could well result in infection of the embryo. Many others also, on the basis of experimental work and the study of human material, concluded that there must be recognizable lesions of the placenta to permit transfer from mother to child. The bacillus of chicken cholera, a member of the hemorrhagic septicemia group of organisms, causes hemorrhages in the placenta in animals and is trans-

mitted to the embryo (Malvoz). Tuberculosis occasionally, and syphilis very often, attack the placenta in man. In a number of instances fetuses have been born with the eruption of smallpox derived from the mother and the transfer of typhoid bacilli through the placenta has been observed occasionally. Similar transmission in man has been noted in relation to anthrax, pneumonia, recurrent fever, and infections with the pyogenic cocci. Abortions, due either to infection or intoxication, may occur during most of the acute febrile infections.

Contrary to the view expressed above, Baumgarten, Birch-Hirschfeld, Lubarsch and many others conceived that a pre-existing injury of the placenta is not essential for transfer; that the micro-organisms, especially when present in the blood in considerable numbers, as in anthrax, may "grow through" the placental vessels in the absence of, and without causing, anatomical lesions.

It is extremely probable that both views are correct, but perhaps in relation to different types of micro-organisms. It has been demonstrated many times, clinically and experimentally, that tubercle bacilli will pass through the intestinal mucosa into the adjacent lymphatics without causing lesions of the mucosa, and, although the conditions are not identical in the two structures (migration of wandering cells through intestinal wall!), the occurrence in one suggests its possibility in the other. However, the existence of the phenomenon is so thoroughly established as to render the exact mechanism a more or less secondary matter.

Latent Hereditary Infection.

It is an important theory of Baumgarten's that tubercle bacilli which are acquired during fetal

life may remain latent until puberty and then, when the unusual resistance which is coincident with rapid growth has subsided, the bacilli multiply and tuberculosis manifests itself. He supposes also that an intermediate generation may, without showing tuberculosis itself, transmit the disease to the next generation (*Uebersprингung von Generationen*). This would seem to presuppose the occurrence of germ-cell infection, but perhaps not necessarily so. As having a possible bearing on Baumgarten's hypothesis, it has been found by Harbitz, by Wechselbaum and others that tubercle bacilli, particularly in children, may exist in the lymph glands without causing anatomical changes. This view has several strong supporters, and it is thought that the bacilli may remain latent in any portion of the body. That hereditary syphilis may remain latent for many years is well known.

On the other hand, it is more generally believed that tuberculosis in most instances is a postnatal acquisition (Koch, Cornet and others) and rational prophylaxis naturally must be based on this conception. Extensive involvement of the liver and periportal lymph glands is characteristic of the placental transmission of tuberculosis. The belief is occasionally expressed that leprosy may be inherited, possibly through placental transmission, but, in view of the non-susceptibility of animals and failure to cultivate the bacillus, the question cannot be taken up experimentally. The possibility of infection of the embryo directly from the father during coitus is discussed, but there is no definite proof of its occurrence.

It is conceivable that micro-organisms may pass from the mother to the child by way of the blood through the placenta at the inception of labor during an early stage of separation of the placenta from the uterine wall.

Infection of a child, as with gonorrheal ophthalmia, during delivery is not regarded as an example of "inheritance" of disease. It is an extra-uterine process, a congenital infection.

CHAPTER VI.

SOURCES OF PATHOGENIC MICRO-ORGANISMS
(Concluded.)

(6) *Dissemination and Transmission by Insects.*
A. Dissemination.

Role of Insects.

The demonstration that insects may play a rôle in the transmission and maintenance of infections dates from the work of Smith and Kilbourne, which disclosed the relation of the tick *(Margaropus annulatus)* to Texas fever in cattle.

Insects may act simply as disseminators of virus, or as the agents of actual inoculation through their bites—i. e., as transmitters. It is important to keep this distinction in mind.

Biologic and Mechanical Transmission.

In their rôle as pure disseminators they may carry micro-organisms from one point to another on their feet, mouth parts, or in their intestinal contents; or, they may act as temporary hosts, the microbes proliferating in their intestinal tract and subsequently being deposited with the feces, in increased numbers, at some new point. The first is pure mechanical dissemination, whereas in the second a biologic factor enters, that of proliferation, and it may be spoken of as biological dissemination. For example, when flies carry typhoid bacilli or cholera vibrios from feces to food, on their feet or mouth parts, or tubercle bacilli from sputum to food or other objects, this is, of course, a purely mechanical process. It would be complicated by the biological feature, however, in case the or-

ganisms, after ingestion by the fly, then multiplied, and were deposited in larger numbers in fly-specks. It is, indeed, a difficult point to determine whether or not proliferation of these microorganisms actually takes place in the intestines of the fly. Observations by Spillman and Haushalter, Hofmann, Celli, Hayward, Lord and others have shown conclusively that house-flies ingest tubercle bacilli from the sputum of patients and excrete them in their feces. The observations of Lord[1] suggest, but do not actually prove, that the bacilli multiply in the intestines. It has been demonstrated several times, by inoculation into guinea-pigs, that the bacilli in the fly-specks are virulent. The specks dry quickly and there is little danger of the organisms taking part in dust infection unless the specks are violently dislodged, and they die in a few days. On the other hand, there may be a real danger from the deposition of the specks on food (Lord), which suggests a point in prophylaxis.

Similar questions are raised also in the relation of the fly to typhoid, cholera, dysentery and plague. The importance of the fly in the mechanical dissemination of typhoid bacilli, resulting in extension of epidemics, is now well established, as illustrated by the observations of Alice Hamilton, and the inquiry into the prevalence of typhoid fever in the American troops during the Spanish-American war. The conditions are similar in relation to cholera. Concerning dysentery it is considered probable that flies are a factor in distribution, although the point has not been positively demonstrated. It is uncertain whether the organisms of

1. Reports of the Massachusetts General Hospital.

typhoid and cholera proliferate in the intestines of the fly. It seems not unlikely in the case of typhoid, inasmuch as Ficker[2] found them in the fly twenty-three days after a feed on the bacilli. Tsuzuki[3] cultivated cholera vibrios from flies which were taken in the dwellings of patients.

It seems probable that flies, in some instances, may distribute plague bacilli after they have themselves become infected by feeding on the sputum of pneumonic patients or on the cadavers of rats dead of the disease. Yersin[4] found plague bacilli in flies dying in a laboratory in which plague was being studied, and in Nuttall's[5] experiments they became infected by feeding on diseased organs.

The Flea and Plague.

The flea, on the other hand, appears to act both as a disseminator and as a transmitter of plague. The human flea, at least two species of rat fleas, and the flea of the dog and cat readily become infected by feeding on rats during the stage of septicemia in the latter, the bacilli multiply for a few days in their stomach and intestines, and are excreted in large numbers in their feces. They are able to communicate the disease to other animals by biting for at least three days after their infection, but in a short time the bacilli disappear from their alimentary tract and they lose the power of transmission. The organisms, which are excreted in their feces, are virulent, and are able to produce infection through very small abrasions and through the minute wounds made by the bites of these insects. The deposition of bacilli in the feces of the flea on the skin of a person is a question

2. Arch. f. Hyg., 1903, xlv, 247.
3. Arch. f. Schiffs-u. Tropenhygiene, February, 1904, viii
4. Ann. de l'Inst. Past., 1904, i, 662.
5. Centralbl. f. Bakteriol., 1897, Abt. I, xxii, 87.

of dissemination, whereas the injection of the organisms through the proboscis is one of inoculation. The flea appears not to undergo a generalized infection with the plague bacillus.

It is possible that rats may become infected with plague also by the eating of fleas which contain bacilli, although this has not yet been demonstrated. Experimentally the disease has been produced in rats by feeding them infected tissues or cultures.

The Bedbug.

Verjbitski also found that the bedbug behaves in a manner exactly similar to the flea in the case of plague. In actual epidemics, however, it seems probable that this insect would be concerned only in the transmission of the disease from man to man, and not from rat to man.

With the exception of the last paragraph the above concerns the mere dissemination of microorganisms by insects. As noted, the diseases concerned are bacterial in nature rather than protozoan. Pathogenic protozoa may be excreted by insects, but, if so, the event appears to be without practical significance, either because the organisms are not viable when excreted, or are not in a stage of development to render them infective, or, what is more probable, that they find no infection atrium when in this condition. Those protozoa which are transmitted by insects usually require actual inoculation in order that they may cause infection.

B. Transmission

Our knowledge is by no means complete on the subject of insect transmission, as a whole, although the essential facts have been worked out in a number of instances, as in Texas fever, some

other piroplasmoses, and in malaria. In some instances it is the micro-organism which is unknown (yellow fever, dengue), in others the question of inheritance in the insect (trypanosomiasis), in others the relation of the insect and the disease to hosts other than man, etc.

Diseases Transmitted by Insects.

Diseases which are transmitted habitually, or mainly, by insects sometimes possess rather distinctive epidemiologic features. Malaria occurs in swampy regions in which certain species of *Anopheles* abound. The distribution of yellow fever, and indeed of all the insect-borne diseases, coincides with that of the insects which are concerned in the distribution. In the temperate and subtropical countries such diseases tend to prevail in the warmer months and to disappear on the advent of frost or cooler weather, an event which is correlated with the activity or inactivity of the insects during these seasons. The Indian plague commission finds that an epidemic wanes when the mean daily temperature is below 50° F., presumably because the flea does not become infected so readily under this condition: the rats die before the advent of intense septicemia, and without the latter the flea is less likely to become infected. The flea may also be less likely to feed generously in the cooler weather. Also it was found that a mean daily temperature of 85°-90° F. coincides with the decadence of an epidemic, and in harmony with this it appears that the flea remains infective for a much shorter time at this temperature. When virtually all the rats of a locality have either been killed by plague, or have recovered from it, those which remain are for the most part immune, and the conditions for a recrudescence

of an epidemic will not be ripe until a new generation of rats has been bred. The immune rats do not harbor plague bacilli, and hence cannot infect fleas. Rocky Mountain spotted fever prevails only in the months of spring. At this time the tick which acts as transmitter is in its adult stage and readily feeds on man as well as on other animals. The larval and nymphal stages appear at other seasons, and, although their bites are infective, they rarely feed on man, either from lack of opportunity or because of a preference for other hosts during these stages. The observation of Carter, that when yellow fever patients are first imported into a new district a definite period (two to three weeks) elapses before new cases develop, suggested some novel mode of transmission, which eventually was proved true when Reed, Carrol and Agramonte proved by experimentation the correctness of the mosquito theory of Carlos Finlay and worked out the details of transmission.

For some time it was supposed that insects transmit only those diseases which are caused by protozoan organisms. This impression arose from the fact that the first examples of definitely proved insect transmission concerned protozoan diseases, as Texas fever (a piroplasmosis) of cattle, malaria of man and birds, and more recently the trypanosomatic diseases. It is only since the relationship of the flea to plague, of the tick to the South African tick fever of man, and of other mites to spirilloses of animals that the importance of insects in transmitting bacterial diseases has been recognized.

Contagiousness Simulated by Insect Transmission.

It is an interesting fact that contagiousness is sometimes simulated in a disease which is trans-

mitted only by insects. Thus, for many years, yellow fever was held to be so contagious that not only direct transfer from person to person was admitted, but also through various indirect means as by fomites. Typhus fever has often been cited as the most contagious of all infections, yet modern studies point rather strongly to an exclusive insect transmission (perhaps fleas or bedbugs). The conditions in plague seem to be somewhat more complex, in that both insect transmission (fleas) and contagiousness prevail, the latter coming into play in the pneumonic form of the disease. Dengue, which spreads like wild fire, possibly is transmitted only by the bites of certain mosquitoes.

In some instances the rôle of the insect is an obligate one—i. e., transmission can occur in no other way than by its bite. This is pre-eminently true of malaria, which, virtually, is incapable of transmission from person to person even when malarial blood is injected into a healthy person. The parasite when it leaves the body becomes infective for man again only after it has completed a sexual development in the mosquito. In some instances infection may be carried from person to person or animal to animal by the injection of diseased blood, yet under natural conditions the rôle of the insect is an obligate one (yellow fever, sleeping sickness, Rocky Mountain spotted fever). The flea in plague transmission is an example of a facultative rôle, since, as stated, this insect is not the only natural means by which the disease is conveyed from person to person.

Sources of Infection.

Insects which carry and transmit infections naturally must have some source from which they

derive the micro-organisms. Commonly, the transmission occurs only between different members of the same species: the insect obtains the virus from one individual and inoculates it into another. In so far as is known, man alone suffers from yellow fever, and the human type of malaria, and he constitutes the only source of infection for the mosquitoes which are concerned in the maintenance of these diseases. The same conditions appear to prevail regarding piroplasmosis in cattle (Texas fever) and in other animals, and also in the South African tick fever of man (a spirillosis), diseases in which ticks are transmitters. Presumably this is also true of some other insect-borne diseases among animals, as the spirillosis of fowls and geese.

In some other instances the existence of a third host has been demonstrated. Man is an important source of infection for the flies which carry sleeping-sickness, but the evidence is strong that some of the native animals of Africa also harbor the trypanosome concerned and that tsetse flies become infected from them as well as from man. In Rocky Mountain spotted fever man is virtually a negligible factor for the infection of the ticks. The circumstances indicate that one or more species of small, wild animals, of demonstrated susceptibility, are the means of keeping the diseases alive in the ticks. It appears to play back and forth from tick to animal, and it is only occasionally that an infected tick becomes attached to man. Fleas probably derive the micro-organisms of plague from rats, in large measure, but experiments also show that they may become infected from man.

Aside from the sources mentioned, the possibility also exists, in relation to some infections, that the viruses are native to the insects, or, rather, that they are habitual parasites in them, just as the colon bacillus is a constant inhabitant of the intestinal tract of man. Even in this case, however, we must assume either some extraneous source for the organisms or that they are inherited from the preceding generation of insects. The latter, indeed, is a method of acquisition which has been shown to occur in ticks, in relation to the Texas fever of cattle, and the South African tick fever, and Rocky Mountain spotted fever of man. The eggs of infected females contain the respective micro-organisms and the larvæ which hatch from them are infective.

Factors in Maintenance of Disease.

The factors which enable an insect-borne disease to be maintained from year to year vary a good deal in different cases. Chronicity, in either the animal or insect host, or in both; inheritance of the disease in the insect, and a rapid alternation of the infection between the insect on the one hand and the animal host on the other—these seem to be the important conditions which have a bearing on maintenance in one disease or another, and all of them assure a more or less constant source for the fresh infection of the carriers.

Texas fever of cattle and other piroplasmatic infections, malaria, and sleeping-sickness are chronic infections in both the animal and insect hosts. Each exists as a more or less protracted source of infection for the other. In addition, Texas fever, and some other piroplasmoses, are hereditary infections in the tick-carriers, and in this way infection is readily kept alive from one

season to the next. It is not yet known whether sleeping-sickness is inherited in the tsetse fly. Malaria is not so transmitted in the mosquito.

South African Tick Fever.

There may be varying grades of chronicity in both the animal and insect hosts. Thus South African tick fever of man (a spirillosis) is semi-chronic, consisting of several recurrences followed by recovery, and it is probable that the tick may acquire the disease from the patient during any one of the recurrences. In the tick, however, the disease is chronic and hereditary.

In these cases the method of maintenance is clear, and in the presence of a sufficient number of insects the conditions are favorable for a thorough infection of the inhabitants. Thus it is that in many tropical districts there are none who do not fall victims to malaria sooner or later.

Rocky Mountain Spotted Fever.

In Rocky Mountain spotted fever we have an example of a condition in which the disease is acute in the various animal hosts, including man, but chronic in the carrier, the tick. In order that fresh ticks may acquire the disease it is necessary for them to feed on a susceptible animal in company with infected ticks, or shortly following a feed by the latter. Since several hundred larvæ or nymphs may be found on one of these animals at the same time, it is readily seen how this may be accomplished. While certain of the small animals (ground-squirrel, ground-hog, rock-squirrel, and perhaps others) are in hibernation the virus still lives in the eggs, larvæ and nymphs; and when the animals "come out" in the spring certain of them become infected through the bites of the larvæ or nymphs and then are in condition to infect fresh ticks. Hence the disease is kept

alive through inheritance in the tick during the months of winter, and at other times through alternation from tick to animal and animal to tick. Man plays little or no rôle in its maintenance, and his occasional infection through the tick bite may be regarded, in a sense, as an unessential incident.

Yellow fever, again, illustrates the condition of an acute infection in the animal host (man) and a chronic in the insect *(Stegomyia calopus)*. Like Rocky Mountain spotted fever, the virus of yellow fever is transmitted in a hereditary manner to the next generation of mosquitoes (Marchoux and Simond) in some instances at least.[6]

Plague. The conditions in plague are peculiar in that the disease runs an acute course in both the animal hosts (rats and man) and in the insect transmitter (flea). Rats do indeed suffer from chronic plague in some instances, but this is not a septicemic condition, hence it affords little or no opportunity for the infection of fleas.

It may be questioned whether the presence of plague bacilli in the stomach and intestines of the flea constitutes a true infection, but it seems justifiable to take this view of the condition, since the bacilli apparently proliferate in this locality for a few days at least (Verjbitski). Verjbitski found that fleas will transmit plague for three days after their infection, the Indian plague commission for from eight to twenty-one days depending on the temperature at which the insects had been kept—for twenty-one days at 75°-80° F., for eight days

6. The British commission also appears to have been successful in proving this hereditary transmission, although the attempt had failed in the hands of Reed, Carrol and Agramonte, and of Rosenau and Goldberger. Possibly it occurs in only a small percentage of the insects.

at 90° F. These findings also correspond with the bacteriologic examination of the intestinal and stomach contents. Verjbitski's work in relation to the bedbug and plague was referred to above.

Although plague is acute in both the animal and insect hosts, maintenance is facilitated through the large numbers of both hosts which are present in plague centers. The conditions render possible a more or less permanent source of infection for the fleas through the continued infection of fresh rats. The possibility also exists that the chronic nodular plague of rats may be subject to exacerbations, accompanied by septicemic infection, a condition which would afford opportunity for the further infection of fleas. It has recently been shown by the work of Wherry and others that the California ground-squirrels have played a part in the persistence of plague in that region, a discovery which may have profound epidemiologic importance for the United States.

Inheritance in the insect has been mentioned as a factor in the maintenance of Texas fever, African tick fever of man, yellow fever and Rocky Mountain spotted fever. Schaudinn also found that the mosquitoes which carry *Trypanosoma noctuæ* (the "halteridium" of the stone owl) pass the micro-organisms to the next generation through the eggs. In Texas fever this appears virtually to be the *sine qua non* for maintenance in view of the peculiar habit of the tick of going through its various stages of development on the host to which the larvæ first become attached.[7] In order to make

7. Most ticks pass through three active stages in their development. The freshly engorged and impregnated female, after a period of rest, lays a great many eggs. Following an incubation period 6-legged larvæ emerge from the eggs, and

this point clear, let us assume that the disease is not hereditary in the tick. Let some normal larvæ become attached to an infected steer. They might acquire the disease from this animal, but could play no part in the infection of others, since they do not abandon this host until they have reached the adult stage and the females are prepared to lay eggs. The latter then drop and lay their eggs, and, in accordance with our assumption, the larvæ which emerge would not have the power of infecting further animals. In this case the tick would be a factor in maintenance only in the event that infected individuals should through accident become dislodged from one host and subsequently become attached to a susceptible animal. This may occur in some instances.

Immunity.

East Coast Fever.

The conditions are different in the case of another piroplasmosis of cattle, namely, the Rhodesian or East Coast fever, which occurs in Africa. In this case, as determined by Lounsbury and by Theiler, the brood from an infected female is not infective, but larvæ, when fed on diseased blood, are infective after reaching the nymphal stage, and, likewise, when infected as nymphs the adults have the power of transmission (*Rhipicephalus appendiculatus*). This is known as stage-to-stage infection, and is important in this instance, inasmuch as the larvæ and nymphs leave the hosts to molt. Following the molt they fre-

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soon become attached to hosts. Then they feed, pass into a quiescent stage, and leaving a white skin, appear as 8-legged nymphs. These also feed, become quiescent for a period, and casting off a white skin are now adults, which are differentiated sexually. In some instances, as in the tick transmitting Texas fever, both molts occur on the host. More frequently, however, other species leave the host to molt. From several weeks to several months are required for the whole cycle.

quently reach susceptible hosts, and thus keep the disease alive. Koch, perhaps in dealing with another species of tick *(Rhipicephalus decoloratus),* found that hereditary transmission of Rhodesian fever does occur.

Piroplasmosis of the dog presents still another interesting condition in regard to inheritance in the tick. Neither the larvæ nor the nymphs of an infected female are able to produce the disease, but when they reach the adult stage they become infective. It has been assumed that the period between the egg and the adult stage is required by the micro-organism for the completion of its sexual evolution, the latter being necessary before it could again become infective.[8] This tick also leaves its host to molt, hence the adults are able to infect new hosts, and from the latter fresh ticks may again become infected. The disease is frequently chronic in the dog as well as in the tick. Motas found that the tick *Rhipicephalus bursa,* behaves in a similar way in the transmission of piroplasmosis of sheep. In this instance the tick remains on the host for the first molt, but leaves it for the second. An infected brood is able to transmit the disease only after it reaches the adult stage.

Christophers has reported still another variation in the inheritance of piroplasmas, in the case of *Piroplasma canis* in India. The tick concerned in this instance was *Rhipicephalus sanguineus,* which leaves its host for both molts. The larvæ of an infected brood are not virulent, but become so when they reach the nymphal and adult stages.

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8. Experiments by Lounsbury with the tick, *Hæmaphysalis leachi.*

Rocky Mountain spotted fever not only is inherited in the tick, but both larvæ and nymphs may acquire the disease by feeding on infected blood and transmit it by biting when they reach the subsequent stage. Hence both hereditary infection and stage to stage infection occur in this case.

It is questionable whether any disease can be maintained in an insect indefinitely through inheritance alone. Mollers[9] found that South African tick fever could be carried into the third generation of ticks *(Ornithodoros moubata)*, neither the second nor third generation of ticks having had opportunity to suck diseased blood in the meantime. The experiment was not carried beyond this point. In relation to Rocky Mountain spotted fever not more than 50 per cent. of the infected females transmit the infection to their offspring, hence maintenance through inheritance alone is considered as impossible.

Certain considerations relative to the course of infection in insects, the incubation period which may intervene between the moment of their inoculation and the appearance of infectivity, and, in addition, the duration of their infectivity, are of interest and importance. These points may be discussed briefly and only in a general way.

Innocuousness of Disease Germs to Insect Carriers.

It is striking that diseases which are transmitted by insects appear to have little pathogenic influence on the insects themselves. The malarial plasmodia penetrate the wall of the stomach, reach the body cavity and eventually the salivary glands, and this, it would seem, without compromising seriously the health of the mosquito. The flea

9. Ztschr. f. Hygiene, 1907, lviii, 277.

harbors in its alimentary tract for several days or weeks the most virulent plague bacilli, but eventually they are cleared away. In those instances in which hereditary transmission occurs, as in piroplasmosis, South African tick fever, and Rocky Mountain spotted fever, the eggs may contain large numbers of the specific micro-organisms and yet give rise to apparently healthy larvæ, which are able to pass through the successive stages into the adult form, still retaining the virulent micro-organisms in their organs and cells. It is probably by virtue of this bland relationship that insects are able to figure as the carriers of infection. If the latter were more virulent for the insect hosts it is fair to assume that their death in large numbers would minimize their rôle as inoculators of other animals.

In the case of the flea and plague, the insect is infective immediately or soon after it has ingested blood, but the period of infectivity is limited to a few days (Verjbitski) or at the most a few weeks (the Indian Plague Commission). This has been referred to above as acute infection of the insect. Mechanical transmisssion is a term which has been used to signify the pure mechanical conveyance of micro-organisms by an insect from a diseased to a healthy animal. The rôle of the flea in carrying plague may be simply a mechanical one, particularly when it transfers the disease immediately after its infection. But, inasmuch as proliferation of the bacillus seems to occur in the flea, and since his infectivity may last for three weeks or more, proliferation may be responsible for the later infectivity. In this event the transmission depends on biological as well as

Periods of Infectivity.

Mechanical and Biological Transmission.

mechanical factors. Transmission of this type probably demands a high degree of virulence and infectivity on the part of the micro-organisms, necessitating the introduction only of very small numbers. One of the conclusions of Verjbitski is that "animals could not be infected by the bites of fleas and bugs which had been infected by animals whose own infection had been occasioned by a culture of small virulence, notwithstanding the fact that the insects may be found to contain abundant plague microbes."[10]

Another condition is represented in Rocky Mountain spotted fever, in which the insects are infective not only immediately or soon after sucking diseased blood, but also for an indefinite subsequent period. In this instance the tick undergoes generalized infection, as previously mentioned. It is possible that the conveyance which takes place immediately after infection is of the mechanical type, but this cannot be true of the later transmissions, nor of those by the members of the following generation. In the latter we have to do with biological transmission, which in these two cases depends on proliferation of the micro-organisms and a general invasion of the tissues of the insects. The term "biological transmission" was originally applied to those cases in which the micro-organisms (protozoan) undergo a complicated sexual cycle of development in the insect, as in malaria, but the principle remains the same with the bacterial diseases, although the method of proliferation is a simple one, consisting merely of fission.

10. Jour. Hygiene, 1908, viii, 205.

Incubation Period in Insects.

When the mosquitoes of malaria and yellow fever suck diseased blood a definite incubation period must elapse before they are able to convey infection to other individuals by their bites. Following the ingestion of malarial blood by the mosquito (various species of *Anopheles*), the parasite undergoes a sexual type of proliferation in the insect's stomach, the product of which, the sickle-shaped bodies, reach the salivary glands only after eight or ten days. These are the pathogenic forms of the parasite which are inoculated into man by the mosquito. In the intermediate stages of this cycle the parasite is not infective. The mosquito *(Stegomyia)* which transmits yellow fever is not infective until at least twelve days after it has sucked diseased blood. And it now appears also that a similar incubation period occurs in the tsetse fly in its rôle as the carrier of sleeping-sickness. After the insects are once infected, however, they continue virulent for a long time. Hence a primary non-infective stage is succeeded by a prolonged infective stage in this type of transmission.

On the basis of the conditions in malaria the temptation has been a strong one to conclude that a primary non-infective stage—incubation period—in the insect carrier is indicative of a sexual cycle in the development of the parasite; hence also of the protozoan character of the parasite. Thus it is anticipated in many quarters that the micro-organism of yellow fever, at present unknown, will prove to be a protozoan, because of the incubation period in the mosquito, mentioned above. Novy, however, has very aptly pointed out that an incubation period in the insect might well occur in the case of a bacterial disease as well as

Transmission of Bacteria.

in a protozoon.[11] Given a case in which the disease is caused by a bacterium rather than a protozoon, we may assume, first, that the infection in the insect is limited to its alimentary tract; or, second, that a generalized infection of the carrier takes place with a localization of the micro-organisms in its salivary glands. In the first instance a large proportion of the organisms ingested with the infected blood reach the stomach and intestines, whereas it is probable that a relatively small number remain in the proboscis. When the insect feeds at once, or soon, on another (healthy) animal the number of micro-organisms injected from the proboscis may be insufficient to infect the new host. Some days might be required for proliferation to result in an infective quantity, either in the proboscis, stomach, intestines, or by contiguous extension, in the salivary ducts and glands. At the time of inoculation the micro-organisms might simply be washed from the proboscis into the cutis by means of the salivary secretion, or directly in the latter, or after a certain amount of regurgitation from the stomach had taken place into the proboscis.

In the second case, in which the insect undergoes a generalized infection, the primary non-infective period (incubation period) may well represent the time required for this general invasion, with the consequent localization of the bacteria in the salivary glands. We have a very good analogy in typhoid fever in man, in which a very definite incubation period precedes generalized infection.

11. The Rôle of Protozoa in Pathology; Proc. of the Path. Soc. of Philadelphia, 1907, pp. 1-27.

Hence it seems possible that errors may result in assuming that a primary non-infective period in the insect points, in an obligate manner, to the protozoan nature of a parasite.

Types of Transmission.

So far we have considered three types of transmission by insects; the first, in which the insect is infective immediately or soon after ingesting diseased blood but not for a prolonged period (the flea in plague); the second, in which the carrier is infective almost immediately and for a prolonged period later (ticks in Rocky Mountain spotted fever); third, in which a harmless incubation period is followed by a prolonged virulent period (malaria and yellow fever).

A fourth possibility, which is perhaps not yet thoroughly established, is that of an immediate infective period, followed by a non-infective, followed again by an infective. The conditions as ascertained so far indicate that this may be the case in the transmission of trypanosomes by tsetse flies. Until lately it was the experience that the flies could convey nagana only for one or two days after their feed on infected blood. Lately, however, Kleine[12] has found that an incubation period of about twenty days, or a little less, occurs in the fly, and from then on, even up to eighty-three days (Taute), it is able to transmit infection. This work has been done with the flies, *Glossina morsitans* and *G. palpalis,* in connection with the trypanosomes of nagana *(T. brucei),* and sleeping sickness *(T. gambiense).* The results have been corroborated by Bruce. The first stage of infectivity of the flies then remains for explanation.

12. Deutsche med. Wchnschr., March 18, May 27 and July 22, 1909.

It is possible that this is purely a mechanical transmission. In many of the experiments rather large numbers of flies have been used on a single animal, and in this case an infective quantity of the parasites might well be introduced mechanically, whereas a single fly might be able to produce infection only after the parasite had multiplied in its tissues, and, perhaps, had reached the salivary glands. It is well known that an intermediate "insect stage" of the trypanosome is not required for infectivity, as appears to be the case with malaria. Trypanosomatic diseases may be carried directly from one animal to another by the injection of blood for an indefinite period. A sexual stage of the trypanosome has not yet been shown to occur in the tsetse fly.

Unique Type of Insect Transmission.

A unique type of insect transmission was described recently by Miller[13] in the case of a disease of rats which is caused by a protozoan organism, *Hepatozoon perniciosum,* a new genus as well as a new species. The transmitter is a mite, *Lelaps echidninus.* The latter derives its infection by sucking the blood of diseased rats, in which the micro-organisms, at a particular stage of their development, occur within large mononuclear leucocytes. A sexual phase of the parasite takes place in the stomach of the mite, forming an ookinet. The latter penetrates the stomach wall, reaches the body cavity of the mite, and becomes encysted (oocyst stage). Within the cyst the parasite subdivides to form a number of sporoblasts, each of which eventually contains from fourteen to twenty sporozoites. Miller readily produced the disease

13. Bull. No. 46, Hygienic Laboratory, U. S. Public Health and Marine-Hospital Service, Washington, D. C.

in many rats by feeding them bread which had been mixed with crushed infected mites. The intestinal juices of the rat break up the sporocyst, and set free the sporozoites, which as "vermicules" penetrate to the veins and lymphatics and reach the liver, where they undergo an asexual proliferation within liver cells. From these the young merozoites are liberated and reach the general circulation, again as vermicules. It is the last stage which is taken up afresh by the mites. Although the sporocysts may occur in the salivary glands of the mite as well as in other parts of the body, Miller found no reason to believe that the parasite is inoculated into the rat by the bite of the mite. The latter feeds only at night, leaving the host in the day, and at a subsequent feed may well reach a healthy rat, which in turn becomes infected by eating a sufficient number of the mites. Miller reproduced the infection also by this "natural" method. Other modes of infection, artificial in character, proved to be unsuccessful.

Insects and Multicellular Parasites.

That insects may have a relation to the maintenance and extension of some diseases caused by multicellular parasites is illustrated by the tropical and subtropical disease of filariasis, of which elephantiasis is one of the most pronounced clinical symptoms. The larval worms often exist in large numbers in the blood of man, particularly at night. When certain mosquitoes suck infected blood at this time one or more of the worms is ingested, undergo further development, and eventually bore through the stomach wall and reach the breast muscles of the insect. The exact manner in which the parasites are reinoculated into man is unknown. It was supposed by Manson that

many of the female mosquitoes die in the water after they have laid their eggs and that the water thus becomes contaminated with the filaria. The use of such water results in the infection of man through his intestinal tract. Suspicion falls on several species of mosquitoes: *Culex pipiens, C. ciliaris, C. fatigans; Anopheles costalis, A. rossi, A. maculipennis.*

A number of worms which are parasitic in one animal or another are derived from insects, the latter serving as hosts for the larval stages. Infection of the digestive canal originates after eating the insects (See Nuttall, Hygienische Rundschau, 1899, ix, 505).

Specificity in Transmission.

There is a certain degree of specificity in the transmission of a disease by an insect, but this is by no means absolute. In yellow fever it appears to be very strong, inasmuch as only one species of mosquito appears to be capable of carrying the disease *(Stegomyia fasciata).* An opposite extreme is found in the case of malaria, in which at least twenty-five different species of *Anopheles* have been shown to be capable of either natural or experimental transmission.[14] Several species of ticks are able to carry Texas fever and probably other piroplasmoses of cattle. At least two species of ticks carry Rocky Mountain spotted fever *(Dermacentor venustus,* Banks, and *D. modestus.* Banks). Two species of tsetse flies may carry the parasites of either nagana or sleeping-sickness. The carrying power, however, is usually limited to different species under a common genus. As far as observations have gone only *Anopheles* carry

14. Ruge: Kolle and Wassermann's Handbuch d. path. Mikroorg.

malaria, only *Glossina* flies appear to transmit nagana and sleeping sickness, and only *Dermacentor* ticks carry Rocky Mountain spotted fever. An exception is found to this in the case of a spirillosis of fowls in South America. Naturally this disease is transmitted by a species of tick, *Argas miniatus,* but experimentally it may also be transmitted by *Ornithodorus moubata.* The latter, in addition to transmitting the disease just mentioned, may also carry the European relapsing fever, and is habitually concerned in the conveyance of the South African tick fever of man.

CHAPTER VII.

SPECIAL FEATURES OF INFECTION.

(*1*) *Virulence, Toxicity, Etc.*

Pathogenicity, Virulence. The word *pathogenicity,* in its relation to infection, refers to the power of an organism to produce disease, and often to the character of the changes which it causes. *Virulence* in all essential respects is synonymous with pathogenicity, but is used more commonly in describing the degree of pathogenic power which a micro-organism posseses. Thus virulent and avirulent (or non-virulent) strains of the cholera vibrio or diphtheria bacillus are spoken of.

Toxicity. *Toxicity* refers to the poisonous properties of a microbe or its secretions. As a property it is not necessarily associated with the living micro-organisms. The question is still discussed as to whether toxicity and virulence are coextensive, even if they are not identical properties. Undoubtedly, toxicity is one of the factors on which virulence depends, and, from the standpoint of the micro-organism, it may be the sole property. Some organisms of little or no pathogenic or infective power nevertheless possess a protoplasm which is more or less poisonous, as certain aspergilli, penicillia or *Bacillus subtilis.*

Infectivity. *Infectivity,* or infectiousness, relates to the power of a micro-organism to maintain itself and to multiply in a living host. One which is able to

obtain a foothold and to produce disease, even when a very minute quantity has been introduced; or one which, after introduction, is able to proliferate to an enormous degree in a particular host is said to be very infective, or infectious, for this host.

Infectivity is not synonymous, nor is it necessarily coextensive, with virulence or pathogenicity. Thus *Trypanosoma lewisi* for the rat, the malarial parasites for the mosquito, and the virus of Rocky Mountain spotted fever for the tick are highly infective, but have a very low grade of pathogenicity for these hosts. The leprosy bacillus, the spirochete of syphilis, and the tubercle bacillus have marked infectivity for man, but their virulence, as indicated by the slow progressive character of the diseases, may be considered as moderate, although the final event may be tragic enough. On the other hand, certain organisms possess great infectivity and great virulence at the same time, as plague, anthrax and the glanders bacilli (acute glanders) for man, and the trypanosomes of sleeping sickness, nagana and dourine for white mice.

Specialization in Virulence.

The virulence of some micro-organisms is often specialized with regard to particular species of animals. Thus measles, scarlet fever and leprosy seem to attack man only, and no animal can be infected with their viruses. Smallpox and syphilis are less specialized. Smallpox can be inoculated into the ox and to a certain degree into the rabbit, and syphilis into monkeys, and perhaps also into the rabbit, although the latter may not yet be definitely determined. Malaria and yellow fever infect only man and the mosquitoes which carry these diseases. Many other pathogenic organisms are in contrast to those mentioned, in that they are able to cause

disease in a wide range of animals. Such microbes are the pyogenic cocci, the bacilli of tuberculosis, glanders, typhoid fever, cholera, paratyphoid fever, tetanus, diphtheria, plague, certain of the trypanosomes, and many others. There are examples of immunity, however, even to these micro-organisms of rather general virulence, such as that of the alligator and the fowl to tetanus, and of the rat to diphtheria.

In contrast to the examples mentioned above, are the non-pathogenic parasites, which experimentation has shown to have no, or insignificant virulence for any animal whatsoever. *B. subtilis* and *B. megatherium* are cited as representing such organisms. The former, however, is not absolutely without pathogenic power, since it has been observed as the cause of panophthalmitis in a number of cases, and by previously lowering intraperitoneal resistance (by the injection of "aggressins") Weil caused fatal peritonitis in guinea-pigs with this organism.

Standing somewhat above these more or less harmless microbes in pathogenic power are the so-called acid-fast grass bacilli and similar organisms, which are able to produce only slight local changes in animals, and which are soon destroyed by the antibacterial agencies of the host.

Variations. Virulence is a variable factor even with regard to different strains of a given micro-organism. Diphtheria bacilli cultivated from various cases show a rather wide range of virulence, and similar observations have been made with regard to typhoid bacilli, streptococci, pneumococci, tubercle bacilli. plague bacilli and other organisms. With few exceptions, pathogenic micro-organisms tend to lose

virulence when they are cultivated for any length of time on the ordinary culture media.

A common device for the maintenance or increase of virulence is that of passage through some suitable animal. The process consists of the inoculation of the animal with a pure culture, permitting the infection to run its course or to proceed for a number of days, and at this time recovering the micro-organism on culture media from the tissues of the animal. Roux and Metchnikoff modified this technic by placing a culture of the organism in a sealed collodion sac, which is then imbedded in the peritoneal cavity of a living animal. After a sufficient length of time the sac is removed and the process repeated. By repeating the passage at suitable intervals virulence may be maintained at a rather constant high point (cholera vibrio, streptococci, etc.). An effective substitute for passage is sometimes found in the use of a culture medium, which in its constitution approximates that of the tissues of an animal. Thus the virulence of pneumococci and streptococci is retained by cultivation on blood agar or in rabbit or human serum or in ascitic fluid. **Passage.**

Animal passage increases virulence in the most marked manner for the species of animal which is used in the experiment, although some increase is usually manifested toward other susceptible species. In a number of instances passage through one animal results in a decrease of virulence for one species and an increase for another. Thus Pasteur found that passage of the virus of swine erysipelas (*Schweinerotlauf*) through pigeons increased virulence for the swine, but it was decreased for this animal when passed from rabbit to rabbit.

The virus of hydrophobia, when passed through several consecutive rabbits, and that of smallpox when passed through the calf, lose in virulence for man.

Effect of Passage.

The increase of virulence which results from passage has been explained by assuming that all the weaker and less virulent individuals of the culture are killed by the serum and leucocytes of the animal, leaving the more resistant and more virulent. This process of selection is probably an important factor in the change, but it would hardly explain the increase which occurs when an organism is grown on heated serum. It seems probable, as suggested by Eisenberg and others, that as a consequence of residence in the body of the animal the culture becomes immunized against the antibacterial factors (bactericidal amboceptors and opsonins) of the host, which would naturally render the organism a more dangerous one for subsequent animals into which it may be injected. It is even possible, as suggested by Welch and others, that certain elements in the body fluids may stimulate the micro-organisms to a more abundant secretion of toxins and other substances (amboceptors), and that the increased virulence caused by passage may depend on the retention of this power after leaving the tissues of the host.

It is probable that all organisms which are able to live for a shorter or longer time within the tissues of an animal cause anatomic changes and abnormal symptoms sooner or later. This, of course, does not apply to those which live habitually on the cutaneous or mucous surfaces. Those which live on the skin do not reach the interior because of mechanical obstacles, and regarding those which

inhabit the mucous surfaces constantly, it would seem that an adaptation has taken place between such surfaces and the micro-organisms, so that the latter, although often pathogenic, are not able to reach deeper tissues. The least harmful infection of which one could conceive would be one in which the micro-organism disturbed the host in no way, except in so far as it used a certain amount of its substance for nutrition. This would be a case of simple, comparatively harmless, parasitism, which in man, is best illustrated by the organisms which live habitually on the mucous surfaces.

It is possible that harmless tissue invasion finds examples in some of the insects, although this may not be maintained positively. The apparent harmlessness of the organisms of malaria, yellow fever, South African tick fever and Rocky Mountain spotted fever for the insects which harbor them, was mentioned in the preceding chapter. Certain infections do, indeed, run a very chronic and benign course, as the ordinary trypanosomiasis of rats, but they are not without discoverable, or even fatal, effects eventually.

Dosage.

The quantity or dosage of micro-organisms required for infection depends on their virulence and the degree of their parasitic power (infectivity); this varies with different species and also with different strains of the same organism. The anthrax bacillus is very infective and may reach a high degree of virulence, so that a single organism has been known to produce fatal disease in experimental animals. Extremely minute quantities of some of the more virulent trypanosomes are required. The tubercle bacillus, even when most virulent, is hardly so infective; it is said that eight

may produce infection when given intraperitoneally into the guinea-pig, and twenty to thirty when into the rabbit (Wyssokowicz). Many hundreds of the most virulent cholera vibrios and typhoid bacilli are required to produce fatal infection in the guinea-pig. In contrast to the conditions mentioned are various saprophytic organisms which, regardless of the quantity introduced, either do not produce infection at all, or do so only after the resistance of the animal has been lowered artificially.

Among those who are equally exposed to infection in an epidemic of typhoid fever, the escape of many probably is due to the ingestion of a small quantity of bacilli which is insufficient to produce disease. Individual susceptibility, and temporary low resistance, are other factors.

(2) *Types of Infection.*

There are wide variations in the physical relationships which different pathogenic micro-organisms hold to the tissues of the body. This has already been suggested in the discussion of infection atria, in which it was shown that certain organisms have a specific preference for points of primary invasion.

This tendency of a specific relationship to particular tissues is kept up in many instances after the microbes have reached the interior of the body.

Selective Action and Distribution.

The malarial plasmodia enter and destroy the erythrocytes and cause enlargement of the spleen and liver, while other organs are affected to a much less degree as a rule. The pneumococcus has a great affinity for the pulmonary tissue and for endothelial structures; it frequently causes

meningitis and is present in the blood in virtually all cases of pneumonia. The gonococcus, aside from its predilection for the urethra, readily becomes localized in the joints, and occasionally on the valves of the heart. The micro-organism which causes acute articular rheumatism has a specific affinity for the joints and endocardium. It would seem that the virus of hydrophobia attacks the central nervous system almost to the exclusion of other tissues. The spirochete of syphilis, although it may cause changes in any organ of the body, is particularly prone to produce proliferation of the vascular endothelium and subendothelial connective tissue.

Cutaneous Infections.

The fungi which cause pityriasis versicolor, ringworm, barber's itch, erythrasma and favus attack only the cutaneous surfaces. Ringworm in the child is prone to be limited to the scalp, whereas in the adult it occurs more commonly on the smooth skin. In ringworm and pityriasis versicolor only the superficial skin is involved, and in the former the hair follicles and the hairs. In favus and barber's itch the *cutis vera* is often invaded, and in the former healing usually takes place with scar formation. The organisms apparently never become generally distributed in the body, and never, or rarely, cause symptoms of general intoxication.

Skin.

There are many other organisms of more general pathogenic powers which frequently cause infections in the skin and other superficial tissues, although they have no specific relationship to the skin. They are found now in one tissue and now in another, and may in fact invade any organ of the body. Such organisms are the streptococci

(erysipelas), staphylococci (acne, furunculosis), the bacillus of anthrax (malignant carbuncle), the tubercle bacillus (lupus, anatomic tubercle), blastomycetes, and others. It is a rather peculiar feature of tuberculosis and blastomycosis that, given a primary infection of the skin, there is not a marked disposition to the metastatic invasion of deeper and remote tissues. This does occur occasionally, it is true, but it seems that a primary localization in the skin has a tendency to immunize the rest of the body against invasion by these organisms.

Local Infection and Soluble Toxins.

Diphtheria and tetanus, as stated elsewhere, represent another type of local infection, the former involving mucous surfaces, the latter being a wound infection. In these, the organisms do not become generally distributed in the body, or, at any rate, not to a marked and essential degree, but the general intoxication results from the action of specific soluble toxins which are absorbed and distributed through the body by the circulation. The organisms are largely limited to the point of primary invasion or implantation. Their toxins may be obtained free from the bacterial cells in artificial culture media, and such toxins when injected are able to cause the symptoms of the disease.

Intoxication Without Infection.

The bacillus of botulism belongs to the same group as the tetanus and diphtheria bacillus, in that it produces a specific soluble toxin in culture media which is able to cause the symptoms of the disease. The mechanism of pathogenesis is different, however. The toxin which produces the disease has already been formed in the diseased meat by the bacillus before the meat is eaten, and the poisoning results from the absorption of this toxin

through the wall of the intestines. The bacillus itself is believed not to proliferate in the intestines. The condition is one of intoxication without true infection.

Continuous Invasion from a Surface.

In some of the intestinal diseases the cavity of the intestines appears to act as a sort of reservoir in which the organisms proliferate to an unlimited degree, and from which they reach the circulation more or less continuously in large numbers, either in a living or dead condition. This relates particularly to typhoid, paratyphoid, cholera and dysentery. They are primarily surface infections. In the two former, however, the organisms, during the early days of infection, reach the circulation in a living condition in large numbers, and may even proliferate in this situation. In cholera, the vibrios show a disposition to general invasion, but appear to be killed off before they have actually penetrated the intestinal wall. The same condition probably prevails in bacillary dysentery. It is the general belief that the intoxication in these instances comes about through the disintegration of the micro-organisms, as a consequence of which a poisonous protoplasm is set free. This conception has its origin from the facts that it has been difficult or impossible to obtain potent soluble toxins in culture media, and that the bodies of the killed organisms are sufficiently toxic to explain the intoxication. Recent work, however, indicates that a certain quantity of toxin may be produced in artificial cultivation, and it is possible that the conditions in the body are much more favorable for toxin production than are artificial surroundings.

Chronic Localized Infections Without Metastatic Extension.

Certain chronic infections, even when they involve deeper tissues and internal organs, show from the

start a disposition to remain localized, although in the end they may involve various organs, by means of metastases, and in some of them a more or less continuous blood infection may arise. Such diseases are tuberculosis, actinomycosis, blastomycosis (oidiomycosis), rhinoscleroma, sporotrichosis, and perhaps leprosy. They are characterized by the formation of a good deal of fibrous tissue, which tends to limit rapid extension by the formation of metastases, and by a disposition to invade contiguous tissues. New foci may be set up in distant organs by means of metastasis, the blood-stream in the meantime being comparatively free from living micro-organisms. A true septicemic condition may be produced in tuberculosis by the sudden pouring of large quantities of bacilli into the circulation from a cheesy focus which has ruptured into a vessel. This again results in the formation of many minute foci in various parts of the body (diffuse miliary tuberculosis). The conditions are similar in blastomycosis, leprosy and glanders. Rarely metastatic infection with actinomyces is found.

These chronic infections are not the only ones, however, in which new foci may originate by metastasis. A streptococcus infection of the heart valves, or an infected thrombus in some vein, cause "pyemic" abscesses in various organs when infected clots from the original site are set free in the circulation. The foci of infection found in the kidney in typhoid fever, and the involvement of the joints in gonorrhea, are further examples of metastatic invasion.

Secondary Systemic Infections. As already indicated, systemic infection may take place in a secondary and accidental way in

many cases when a pre-existing local disease exists at some point. In a small group of diseases (typhoid, paratyphoid, pneumonia) this secondary general invasion occurs with great constancy, and the organisms can always be cultivated from the blood if this is undertaken at the proper time. In syphilis general invasion occurs only after a certain "incubation period" has been passed in the primary sore, and after this time it exists as a protracted blood infection.

Primary Systemic Infections.

Others appear to be primarily and essentially blood infections, little or no reaction taking place at the point of invasion. This is true of systemic plague, anthrax, Rocky Mountain spotted fever, some very virulent infections with the streptococcus, relapsing fever, and in many of the protozoan infections, as in malaria, piroplasmosis, and in sleeping sickness. In some of these the organisms may pass a portion of the incubation period in the lymph glands, where they proliferate to such an extent that they gradually overwhelm the circulation in large numbers. The organisms of malaria and piroplasmosis proliferate in the blood-stream, i. e., within the erythrocytes. It also seems probable that the other organisms mentioned are able to proliferate in the plasma, and that their presence in the blood-stream is not due entirely to their continuous escape from such solid organs as the lymph glands and spleen, or from the point of primary invasion. They are true or full parasites in the sense of Bail.

From the standpoint of continuity there are several types of systemic infection.

Continuous Systemic Infections.

We may, in the first place, recognize the continuous type, in which the organisms, after they once

reach the circulation, persist in that situation until the infection terminates either in death or recovery, in the latter case being exterminated by the protective agencies of the blood. Typhoid and paratyphoid fever, plague, Rocky Mountain spotted fever, Malta fever, and probably the acute exanthemata, are of this type. Recovery and the sterilization of the blood, however, does not mean that the whole body is necessarily rid of the micro-organisms; the latter still may persist for a greater or less period on one or more of the body surfaces, as in the case of typhoid fever, in which the bacilli may persist in the intestines or the bladder for a long period, or in plague in which the organisms may be found in the sputum for some time. Through some accident typhoid may vary from its usual habit, a point which is illustrated by the occasional occurrence of relapses, or by the localization of the bacilli in some solid organ of the body, as in the vertebræ or the muscles, resulting in post-typhoidal complications.

Periodic Systemic Infections.

In other instances the systemic invasion is of periodic character, and of this there are a number of varieties. The streptococcus, when it exists as the cause of fibrinous endocarditis or of thrombophlebitis, often invades the circulation in a fluctuating manner. Periods when there are very few cocci in the blood will be followed by others in which they are very numerous, and the clinical symptoms usually correspond with these fluctuations. A similar course probably is followed by tuberculosis and blastomycosis in their invasion of the blood. Mechanical factors sometimes precipitate a systemic distribution, such as the rupture of a caseous nodule into a vessel or lymph channel in

tuberculosis, or the separation of minute fragments of thrombus infected with streptococci.

Regular Recurrences.

Examples of a more or less regular periodicity are found in the various relapsing fevers, which are caused by spirilla. The first attack of the European relapsing fever lasts for six or seven days. This is followed by a period of apyrexia of five or six days, followed by another febrile period. Recovery is usually established after three or four such attacks. In the relapsing fever of South Africa the first attack has a shorter duration (about three days), and those which succeed may last only one or two days, according to Koch. During the febrile attacks the blood swarms with spirilla, whereas in the intervals it is comparatively free from organisms. In explanation of this it has been assumed that the febrile attacks are cut short by the development of a certain degree of immunity. This results in a more or less complete sterilization of the blood, although spirilla which remain in the lymphoid organs, particularly the spleen, seem to be protected. At the time of a relapse, either the immunity has decreased sufficiently, or the remaining organisms have gained in virulence to such an extent that a general reinvasion takes place. In the end the immune forces gain the upper hand and the body becomes completely sterilized.

Recrudescences in Syphilis, etc.

Some of the chronic infections are subject to irregular recrudescences. Syphilis and trypanosomiasis of man begin as acute infections. After the acute secondary stage has apparently passed, syphilitics frequently suffer recrudescences, with general manifestations, and it is probable that the number of micro-organisms in the blood increases

at such times. In the first stage of sleeping sickness, the so-called trypanosomatic fever, the fever is of a remittent type, and during the attacks of fever the number of trypanosomes in the blood increases. In the stage of sleeping sickness they appear to be limited very largely to the lymphatic glands and the meninges. They can readily be obtained from these sites by puncture, but their presence in the blood is much more inconstant. It has been the experience that on some days prolonged examination of the blood will disclose no trypanosomes; on other days two or three per field may be found; and on still others as many as seven or eight per field.

Cyclic Invasion in Malaria.

The tertian and quartan infections with the malarial parasites, at least in their early stages, illustrate a special type of recurrent generalized infection, which is cyclic in nature, the period of intense general invasion coinciding with a certain stage of the asexual multiplication of the parasites. (See chapter on malaria.)

(3) *Nature and Mechanism of Infection.*

Penetration by Micro-organisms.

It is to be understood that infection presupposes a penetration of the body surfaces to a greater or less degree. Even in pityriasis versicolor, the most superficial of infections, the fungus penetrates the horny layer of the skin. Hence, in a consideration of the nature and mechanism of infection, it would be desirable, first of all, to consider the manner in which micro-organisms and their poisons may reach the deeper tissues. In some instances this is readily understood, whereas in many others we must in the main be content with mere deductions.

Skin.

Certain animal parasites, as the itch mite and jigger, penetrate the surface through mechanical defects of their own making.

Mechanical Implantation.

In penetrating wounds, abrasions and transmission by insects the introduction of micro-organisms is a question of mechanical inoculation, or subsequent growth into the defect. In this case there is no barrier to their entrance into the circulation, until after the appearance of an inflammatory reaction; and if the organism happens to be one which secretes a soluble and easily diffusible toxin (e. g., tetanus bacillus), general intoxication may result even without further dissemination of the living cells. The degree of defect necessary for infection varies with the character and virulence of the organism. The bacilli of plague, anthrax, glanders, and the spirochete of syphilis may enter through lesions which are almost microscopic in size. These organisms possess great infectivity, exceedingly small numbers producing infection.

Penetration by Contiguous Growth.

Organisms, such as virulent staphylococci, which reach the hair follicles and sebaceous glands, are able to grow through the succulent epithelium into the surrounding tissues, and to cause a furuncle, carbuncle or cellulitis. This may follow or be accompanied by a primary necrosis of the epithelium. Occasionally when furuncles are situated at favorable spots, as near the angle of the mouth or nose, the necrosis may extend to adjacent veins, resulting in a flooding of the circulation with the organisms.

True soluble toxins are seldom absorbed through the unbroken skin, if we except the case of poisoning with poison ivy. In Moro's test for tuberculosis the tuberculin, incorporated in a paste, is

rubbed into the skin. This is also true of most chemical poisons which do not have a corrosive effect, although by prolonged contact (lead) or rubbing (mercury) a certain amount of absorption may be induced.

Mucous Surfaces.

In infections of the mucous membranes, with their soft epithelial covering and moist surfaces, micro-organisms are often able to grow through into the underlying tissue, resulting in either a local inflammation, a general infection through the medium of the lymphatics or capillaries, or a general intoxication through the absorption of toxins. A mechanical defect in the surface may not be necessary for this penetration, the extension taking place by contiguous growth, which, however, is surely favored by any toxic and desquamating effect which the organism or its toxin may have on the epithelium. Streptococci which reach the crypts of the tonsils readily cause necrosis of the surface epithelium, and this defect would seem to facilitate deeper invasion.

Contiguous Growth.

Diphtheria.

The growth of the diphtheria bacillus is limited in the main to the superficial layers of the tissues which it attacks. As the organism penetrates the epithelial layer, possibly by direct growth, the toxin which it secretes causes necrosis of the adjacent cells. This process continues until the vascular tissues are reached, resulting in the formation of a false membrane. However, the onset of fever before the formation of the membrane, and the occurrence of diphtheria without membrane formation, show that the false membrane, i. e., the necrosis of the surface, is by no means a prerequisite for the absorption of the toxin. A severe invasion of the body by the bacillus does not occur, although

occasional individuals, without doubt, reach the circulation; this, however, is not necessary for the general intoxication.

Leucocytes as Carriers.

Leucocytes are continuously passing from the tonsils, intestines and other superficial organs which are rich in lymphatics, through the mucous membrane to the surface. These excreted leucocytes may often be seen engorged with large numbers of bacteria which they encounter on the surface, and it has been suggested that the excreted leucocytes may re-enter the adjacent tissue, carrying bacteria with them. The study of sections of the intestines, tonsils and peribronchial lymph glands has shown that bacteria are continuously entering the body, even in health, and their frequent occurrence within leucocytes which are near the surface suggests that the latter are the agents through which they are introduced (Ruffer, Bizzozero and others). This finding, however, could not be accepted as proof of the hypothesis, since the phagocytosis may have taken place after the organisms had penetrated the surface independently.

Through the work of Nocard, Ravenel, Behring and others, it has been shown that tubercle bacilli will pass into the lymphatics from the intestines, in the absence of mechanical defects.

That the leucocytes may perform this function is also suggested by A. B. Macallum's study of the absorption of iron. When the albuminate and peptonate of iron were fed to starved lizards, the mineral, eight hours later, was demonstrated in large quantities in the leucocytes contained in the lumen of the intestines, also within leucocytes which lay between the epithelial cells of the villi (re-entering

leucocytes (!),and within similar cells which were found in the liver and spleen (Adami's Principles of Pathology, Vol. I, p. 291). Inasmuch as no power of spontaneous penetration can be ascribed to the particles of iron, it is held that they were carried into the tissues by inwandering leucocytes.

It may be stated, then, as a reasonable probability that micro-organisms are sometimes carried into the deeper tissues by leucocytes which re-enter the surface.

Phagocytosis of bacteria by epithelial cells, particularly by the pulmonary epithelium, is known to occur, but it is not known that the process is an essential one for invasion.

Toxins. Some toxins are not readily absorbed by the normal mucous membranes, whereas others seem to be taken up readily. In the absence of wounds, tetanus toxin, when ingested, causes no symptoms. It is destroyed largely by the gastric and pancreatic juices, and this is the case also with diphtheria toxin in test-tube experiments. The latter has the power of causing necrosis of the mucosa, and may be absorbed through the injured surface. The toxin of hay fever is readily absorbed through the conjunctiva and the mucous membrane of the nose. Experimentally, ricin, a plant toxin, is absorbed through the intestines, although the amount required for fatal intoxication by this route greatly exceeds that of the subcutaneous injection. The toxin of the bacillus of botulism is readily absorbed through the intestines of both man and animals.

Denudation of Surface. When a micro-organism causes a primary desquamation of the mucous epithelium, it follows that further penetration of the organism, as well

as absorption of toxins, are facilitated. This would seem to find special application in cholera and bacillary dysentery. In cholera general invasion of the body by the living organisms does not take place, although the intestinal surface is denuded to a greater or less degree. The vibrios penetrate to a certain rather superficial depth, where they appear to become dissolved, and with their dissolution, poisons, in addition to those which were previously secreted, are set free. The conditions are similar in dysentery.

Summary.

To summarize, micro-organisms gain entrance to the subjacent tissues through wounds, by means of their own power of injuring the surface and growing into and through it, and probably also through the agency of inwandering leucocytes. Phagocytic epithelial cells in some instances may play a part, but this is hypothetical. Toxins are absorbed for the most part through surfaces which have been previously injured, but some of them are able to pass through previously healthy mucous membranes.

Incubation Period.

The term incubation period signifies the interval between the first introduction of a pathogenic micro-organism, or its toxin, until the development of the first symptoms which characterize the onset of the disease. In many infections, as in typhoid fever, a feeling of malaise, headache and nausea, frequently appear a few days before marked symptoms develop, and this period is called the prodromal stage. It may be considered either as the latter end of the incubation period, or as the beginning of actual "onset."

Duration.

The length of the incubation period varies greatly in different infections. In cholera it may

be as short as a few hours only, in hydrophobia it is on rare occasions as long as six months, but more commonly two to four weeks. In a rather large group of diseases the onset follows in from one to two weeks after exposure, so that there would seem to be a tendency to some general law, the basis of which is not known.

It has been suggested that it may have a relation to the anaphylactic reaction (Rosenau and Anderson); namely, that the first micro-organisms introduced "sensitize" the body in some way not yet understood, and when sensitization has taken place (seven to fourteen days) the body then has a greater susceptibility to the organism and yields readily to infection (see "Anaphylaxis").

Occasional individuals show a susceptibility to a first injection of horse serum (e. g., diphtheria antitoxin) in that they develop an urticarial rash, adenopathy, effusions into the joints, and perhaps fever, in from eight to twelve days after the introduction of the serum. Von Pirquet noted that when a second injection of the serum was given after an interval of two weeks or more similar symptoms would develop within a few hours rather than after several days; and there is a great deal of constancy in this when susceptible individuals are concerned. He arrived at the conclusion that the horse serum as such is not toxic for man, but that as a consequence of the injection antibodies to the serum are formed and that the intoxication comes about as a result of some kind of chemical reaction which takes place between the antibodies and the serum. The conditions in immunization render this explanation extremely probable. After the first injection of serum antibodies to the serum

(precipitating and perhaps other antibodies) are found in considerable concentration in the blood only after the lapse of some days. If the injection of serum given in the first place was of some size, some of the original serum proteids would still be present in the body when the antibody formation had reached a high point, and the conditions for the toxicogenic reaction between the two would be present. This would account for the delayed reaction seen in first injections. If some smaller quantity were given in the first place the delayed reaction might not occur, because the serum proteids would all have been modified or excreted. If a second injection is given, however, the fresh serum at once comes in contact with the antibodies which have already been formed, and the toxic combination or substance can be produced at once.

On the basis of these considerations von Pirquet believes that the ordinary interpretations of the nature of the incubation period are incorrect. As he says: "I presented the theory that the disease-producing agent only calls forth pathological symptoms in the body when it is changed by means of the antibodies; the incubation period is the period required for the formation of these antibodies." This possibility cannot be overlooked in a present-day consideration of this subject, although it is still on a theoretical foundation.

Relation of Dosage.

The number of micro-organisms introduced has an influence on the incubation period. Guinea-pigs may be killed within a few hours by the injection of a large quantity of typhoid bacilli, but with the administration of smaller quantities a much longer incubation period may be obtained. The effect of quantity also is shown very clearly by the

injection of diphtheria or tetanus toxins, by which the incubation period can be shortened from several days down to several hours, depending on the quantity injected.

The so-called true toxins of bacteria, i. e., those which are able to cause the formation of antitoxins, as a rule are distinguished from other poisonous substances of bacterial or other origin, by the occurrence of an incubation period when they are administered. However, at least two toxins, venom and that secreted by the *Vibrio Nasik* and perhaps other vibros, act so quickly that an incubation period is hardly to be recognized.

Proliferation. The number of micro-organisms originally entering the body is usually quite small; hence the time required for them to reach an infective or toxic quantity probably represents a part of the incubation period.

Mechanical Factors. In hydrophobia and tetanus a mechanical factor plays a part in the length of this stage. In these cases the micro-organisms (hydrophobia) and toxin (tetanus) appear to reach the central nervous system by way of the peripheral nerves, and symptoms are delayed until this occurs. Wounds of the face and neck are followed by symptoms more quickly than when they are situated on parts more remote from the central nervous system.

Adaptation. It is not unlikely that another factor consists of a certain relation between the protective powers of the host and the invasive or aggressive ability of the organism, at least in some instances. Lemaire and also Buxton found that after the injection of pathogenic micro-organisms directly into the circulation, there is at first a reduction in their number, followed by renewed proliferation, which

progresses steadily. Supposedly, the natural antibacterial forces of the serum and leucocytes took up the destruction of the bacteria until the former were exhausted, and from this time proliferation could take place with little hindrance. In infections which occur naturally, as in typhoid fever or scarlet fever, the first organisms which reach the underlying tissues and circulation may be destroyed, involving such a loss of bactericidal agencies that continued proliferation and invasion finally results in general infection.

"Serum Resistance."

In speaking of passage, it has already been stated that virulence may be increased by permitting an organism to grow in the tissues of a living animal. Virulent organisms are not taken up by leucocytes so readily as avirulent, and in the presence of high virulence there may be no phagocytosis at all. This has a bearing on the incubation period in that the micro-organisms which first come in contact with the tissues of the host may have low virulence, but after exposure to the germicidal substances for a sufficient length of time, their pathogenicity and resistance may be so raised that they escape destruction in the tissues. On this basis, therefore, the incubation period may, in part, represent the time required for the organisms to become serum-resistant.

Regarding the subject under discussion, there is still another factor which finds application, particularly where toxins having an enzyme-like nature, are involved. Toxins have been likened to enzymes, because of their action in exceedingly small doses, their common susceptibility to heat, and finally the exhibition of an incubation period. No matter how much diphtheria or tetanus toxin

is introduced into an animal, the incubation period cannot be eliminated absolutely; and some of the hemolytic toxins, as tetanolysin, can be added to red blood cells in test-tubes in any desired quantity without causing their immediate solution. There is, therefore, something inherent in the nature of these substances, or in the nature of the action which they exert on the cells of the body, which demands this latent period before an effect becomes manifest.

Summary. It seems probable, therefore, that there are many factors which contribute to the existence of the incubation period, and that the factors which determine its length in one instance may not be identical with those which are found in another. The time required for proliferation of the micro-organism to an infective quantity probably is common to all infections. The infective quantity must vary with different diseases, and with different strains of the same micro-organism depending on their virulence. A virulent strain has a shorter incubation period than a less virulent. In some instances a certain amount of time may be required for the micro-organism to undergo an increase in virulence sufficient to accomplish infection. Or, again, this time may be required for the exhaustion (absorption or chemical binding) of the protective substances to such a degree that further proliferation and invasion take place with greater rapidity, this latter step coinciding with the onset of marked symptoms. The time required for distribution of the poisons to vital organs would seem to be of minor importance except in hydrophobia and tetanus, which utilize the peripheral nerves as a route to the central nervous system.

In the case of some of the toxins (as of diphtheria and tetanus) a certain time for the manifestation of a toxic effect is required, even when they are placed in direct contact with the cells for which they have a specific affinity. As stated, the rôle of anaphylaxis is uncertain.

Factors in Establishment of Infection.

Leaving out of consideration the few instances in which preformed toxins are ingested and absorbed (as in botulism), the production of disease in a susceptible host would seem to depend on two factors: First, presence in the micro-organism, or secretion by it, of a toxin which is able to cause a direct injury of the tissues of the host; and second, ability of the micro-organism to remain alive and to proliferate in the body of the host.

Direct Chemical Injuries.

Different toxins vary greatly in the cells which they attack. Some destroy the red blood cells to a marked degree (staphylococcus and streptococcus); others have a special affinity for the nervous tissue (tetanus, diphtheria, botulism); some attack particularly the endothelium of the vessels, causing many minute hemorrhages (some of the eruptive fevers, rattlesnake venom). In many other instances the toxins have a wider range of action, and many different tissues suffer to a greater or less degree. Areas of necrosis in the lymphoid and parenchymatous organs, and granular and fatty degeneration of the latter, and of the muscles, including the heart, are well known in different diseases. The albumin and casts which appear in the urine in various acute febrile diseases are a result of a toxic action on the epithelium and endothelium of the kidneys.

In addition to destroying life by their direct action on the cells, pathogenic micro-organisms

produce profound disturbances in metabolism and nutrition by interference with the functions of organs. It is not the intention, however, to enter into a discussion of these obscure influences.

Mechanical Injuries. The mechanical injuries which micro-organisms cause are, at least in most cases, the result of a previous toxic action, i. e., they are secondary effects. This is true of the emboli, consisting of a mixture of fibrin, cells and micro-organisms, which arise in a valvular endocarditis, and of thrombi which are formed in vessels as a consequence of infection.

Fibrin. 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 toxic action of the pneumococcus on the capillary walls and the alveolar epithelium, permitting the escape of the blood and serum.

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

Malaria. A genuine direct mechanical disturbance appears to be caused by the malarial organisms in that they occasionally accumulate in the capillaries of the intestines and brain in such numbers as to

amount to virtual thrombosis. Filariæ, which are multicellular organisms, cause grave conditions by occlusion of the lymphatics.

In discussing the second condition for the occurrence of infection, namely, the ability of the micro-organism to remain alive and to proliferate in the body of the host, this must be done with regard to certain protective agencies which the host possesses against invading micro-organisms.

Anti-infectious and Antitoxic Agencies.

As will appear later in more detail, these agencies may be directed either against the toxins of the micro-organisms, or against the parasitic cells themselves. The former rests in the antitoxins which may be present in the plasma and lymph, and the power of the tissues to bind and destroy the toxins; the latter in the germicidal substances of the body fluids (the so-called bacteriolysins), and in the phagocytic and destructive action of various cells of the body, particularly the leucocytes and endothelial cells. Manifestly, in actual infection the micro-organisms are able to proliferate in spite of the presence of these antagonistic agencies, and the conditions which render this possible probably vary a great deal in different infections.

Natural Resistance of Organisms.

Thus, in relation to tuberculosis, either the serum of man and animals possesses no germicidal substances for this particular organism, or the latter is resistant to their action. Also, since the bacilli are frequently found within phagocytic cells in a good state of preservation, it would seem that they have a certain degree of resistance to intracellular digestion. Staphylococci, streptococci and certain other organisms resist destruction by the serum, although they are readily taken up and destroyed by phagocytes.

Acquired Resistance of Organisms.

This insusceptibility of the tubercle bacillus and the cocci mentioned to the germicidal action of the serum is a natural and constant property. On the other hand, certain organisms which are naturally susceptible to the germicidal power of the serum and leucocytes appear to acquire a resistance against these agencies during the course of infection, and it is not at all unlikely that this is a property which is common to all pathogenic micro-organisms. We may look on this change as a phenomenon of adaptation. In a distinct sense it appears that the micro-organisms may become immunized to the bactericidal substances of the serum, and to the opsonins on which destruction by phagocytosis depends.

The increase of virulence in a micro-organism by repeated passage through a suitable animal may be regarded as an adaptation on the part of the organism for the tissues and fluids of the animal, and in this adaptation an increased resistance to the germicidal substances probably is an important factor. Similar agencies doubtless are at work in the maintenance of virulence by growing cultures on media which contain the serum of animals.

Resistance to Agglutinins.

Typhoid bacilli which have been cultivated from the body of a patient recently are more resistant to agglutination than those which have been on artificial media for some time. The same has been found true of the cholera vibrio (Pfeiffer and Kolle), as well as of some other organisms. Also the cultivation of bacteria (typhoid bacilli) in media which contain an agglutinating serum brings about an increased resistance to agglutination (Ransom and Kitashima, Walker, Steinhardt and others); and it has been shown that such modified

bacilli absorb or bind less agglutinin than "normal" strains (Müller, Cole, Eisenberg). Explained in terms of Ehrlich's theory, it is assumed that they contain a decreased number of binding molecules or receptors for the agglutinin, hence an effective quantity of the latter is not bound.

Resistance to Bacteriolysins.

Experiments by many observers indicate that bacteria may also acquire resistance to the bactericidal action of the serum, and that this property goes hand in hand, at least to a certain extent, with the virulence of the micro-organism. In some of these experiments the resistance has been acquired by growing the organisms in the corresponding antiserum, for a greater or less length of time. Thus Cohn, by growing the typhoid bacillus on antityphoid serum, conferred on it an increased resistance to bacteriolysis, which was retained for several subsequent generations on agar. Day also, in a similar way, caused increased serum resistance in *B. prodigiosus, B. vulgaris* and *B. fluorescens,* and even conferred some pathogenic powers on these organisms, which naturally are rather harmless saprophytes.[1] Such results have been obtained with the organisms of typhoid (Walker, Haffkine and others), cholera (Szekely, Ransom, Kitashima and others), dysentery (Marshall, Knox, Mason), anthrax (Shaw, Danyz and others), the colon bacillus (Hamburger), *Vibrio metchnikovi* (Metchnikoff, Bordet and others). It has frequently been found also that bacteria when freshly cultivated from infections have an unusually high resistance to the bactericidal action of serums. Besserer and Jaffé noted that a strain

1. Eisenberg gives a summary of this and kindred subjects in the Centralbl. f. Bakteriol., etc., xlv, No. 2.

of the typhoid bacillus isolated from a "carrier" may show this increased resistance.

Resistance to Phagocytosis.

It is an old tenet of Metchnikoff that virulent micro-organisms are less susceptible to phagocytosis than avirulent. This was supposed to be due to the establishment of a negative chemotaxis, which consisted either in a repelling of the leucocytes or in their failure to be attracted to the bacteria. In 1892 Massart reported that leucocytes were negative chemotactically to virulent strains of the organisms of anthrax, chicken cholera, swine plague, and to *B. pyocyaneus* and *Vibrio metchnikovi,* and that they were taken up by leucocytes in the animal experiment to a much less extent than avirulent strains of the same organisms. The same condition was noted in relation to staphylococci (van der Velde, Kocher, Taval). Since phagocytosis has been studied more extensively under artificial conditions, the same phenomenon has been noted in relation to other organisms, as the streptococcus (Hektoen and Ruediger, Bordet, etc.), pneumococcus (Rosenow), typhoid and paratyphoid bacilli (Neufeld and Hüne, Hektoen), the colon bacillus (Beattie), the meningococcus (Flexner), and others. In African tick fever, Levaditi and Roché noted the presence of spirillicidal and opsonic substances (those favoring phagocytosis) following the first attack of fever, but they appeared to be effective only on the spirilla which were present in the blood during the first attack. By the time the second attack occurred the organisms had acquired a resistance to these substances which they retained during several passages through rats subsequently. It would seem that this acquired resistance on the part of the organ-

isms is what makes the second attack possible, and that this might go indefinitely were it not that the host eventually responds by the production of such a quantity of germicidal substances that the spirilla are finally destroyed. We may suppose that the conditions are not very different in syphilis and in some of the chronic protozoic infections, as trypanosomiasis and piroplasmosis; in all these diseases specific antibodies are produced during the course of infection, and there is good reason to believe that some of these are germicidal in character. This is certainly true in regard to piroplasmosis and trypanosomiasis, yet the organisms persist in the blood and tissues of the host for a long period. It seems that they must have become adapted to the presence of these germicidal substances. That virulence is not lost as a consequence of this adaptation is shown by the fact that the blood when it is injected into a fresh animal reproduces a typical acute attack.

Acquired Resistance to Drugs.

It is noteworthy that a similar resistance to certain drugs can be induced in trypanosomes when infected animals are given repeated injections of these preparatoins. If a sufficient quantity of one of these drugs (atoxyl, fuchsin, trypan-red, etc.) is given to an infected animal, a complete cure, with sterilization of the body, may be obtained. In the event, however, that an insufficient quantity of the drug is administered, the organisms when injected into a subsequent animal may be entirely indifferent to the presence of the drug, which, consequently, has lost its therapeutic value against this strain. It may be necessary to repeat this process through many passages before a high degree of drug resistance is obtained, but when once estab-

lished it is of long duration. Such strains are called "chemoresistant." It has also been shown that trypanosomes acquire a resistance to the germicidal properties of serum, as in the case of bacteria.

Capsule Formation.

In view of the fact that some micro-organisms produce a capsule when grown in the animal body, whereas it is absent in ordinary culture media, it has been supposed by some that the capsule is an expression of adaptation on the part of the organism to the germicidal agencies of the host, that it is perhaps protective in its character. Such observations have been made in relation to the anthrax bacillus (Deutsch and others), the streptococcus (Bordet, Marchand and others), and the plague bacillus (Löhlein).[2] This relationship, however, is not general, and can hardly be considered as thoroughly established.

Injury of Protective Agencies.

In addition to this more or less passive adaptation on the part of micro-organisms, there is reason to believe that they may actually antagonize the protective agencies of the body, particularly the process of phagocytosis. Arloing and Courmont, and Roger believed that bacteria secrete substances which favor their growth in the body. Roger observed that an extract of the bacillus of symptomatic anthrax when injected into the rabbit favored infection with this organism. Bouchard spoke of such elements as *substances favorisantes.* Kruse (*Zeigler's Beitrage,* xii, 339) also believed that such substances exist and called them collectively *lytische Sustanzen, Angriffstoffe, oder Lysine.* He supposed that their effect was chiefly to neutralize the alexins, the name which, at that time,

2. Cited by Eisenberg, l. c.

was applied to the bactericidal substances of the serum. Their existence had no satisfactory support at that time.

Leucocidin.

A little later van der Velde discovered that staphylococci secrete a substance which is toxic for leucocytes, the so-called *leucocidin*. It appeared to be produced by strains of both high and low virulence. Still more recently it was discovered that the streptococcus produces a toxin which is destructive for leucocytes (Ruediger, Besredka). These results suggest that a toxic action on the leucocytes may be a factor for the progress of infection, and may explain, at least in part, the resistance which virulent organisms show toward phagocytosis. A particularly significant observation is that by Rosenow, to the effect that virulent pneumococci secrete a substance which has the power of inhibiting phagocytosis, a substance to which the name of *virulin* was given. Virulin can be extracted from virulent cultures by means of salt solution, and after this has taken place the cocci have become susceptible to phagocytosis. Also after avirulent strains have been treated with the extracted virulin, and later separated from it by washing, they are found to have acquired resistance to phagocytosis. Eisenberg also observed that leucotoxic substances are produced by the bacilli of symptomatic anthrax and malignant edema. He furthermore believes that it is a more or less common property of bacteria to produce such substances in the course of infection, although they may not be obtained under artificial conditions, and that they are identical with the "aggressins" of Bail. The correctness of this belief, however, is not fully demonstrated.

Virulin.

Aggressins. Bail, discrediting the importance of the germicidal substances of the serum for natural immunity, assigns to phagocytosis the essential rôle, and believes that the virulence of the parasites depends on their power to produce substances, the aggressins, which antagonize the process of phagocytosis. The aggressins were supposed to be non-toxic substances which were produced only in the body of the infected animal. When the organisms of typhoid, cholera, tuberculosis and other diseases, are injected intraperitoneally into the guinea-pig, the aggressins appear in the inflammatory exudate, and are obtained free from living micro-organisms by centrifugation and subsequent chemical sterilization of the overlying fluid. This fluid, when mixed with the homologous culture, has the power of rendering fatal a quantity of the culture which otherwise would be unable to produce infection, and the mixture may cause a very acute death of the experiment animal. Bail found all the more justification for assuming the existence of this new (?) substance from the fact that immunization with the aggressins gives rise to a serum which has the power of neutralizing the effect of the aggressive exudate; hence such a serum was termed an antiaggressin.

Objections. The studies of others, however (Wassermann and Citron, Doerr, Sauerbeck and others) indicate that the aggressins of Bail do not represent a new entity. Wassermann and Citron found that the "aggressins" as prepared by Bail are toxic, and that they may be obtained in artificial cultures as well as in the body of an animal. They probably represent nothing more than the cytoplasm of dissolved bacteria which may exert an inhibiting effect on pha-

gocytosis in more than one way. The toxic substances may injure the leucocytes to such an extent that they do not readily take up the living organisms; since they represent disintegrated organisms, they may, like the latter, absorb or "bind" the opsonins of the plasma, on which phagocytosis depends, and thus prevent the action of the opsonins on the bacterial cells; similarly, they bind the bacteriolysins of the serum, and hence divert their action from the living cells; also, since the dissolved organisms are toxic, when the "aggressins" are added to living cultures, the total toxicity for the animal is increased by just that much. There is also evidence to show that the most indifferent proteid substances when injected into an animal may decrease its resistance to infection or intoxication for the moment. Thus Ricketts and Kirk found that a small quantity of egg albumin, broth, or normal serums (goat, guinea-pig) when injected into white mice, decrease the resistance of the latter to a concomitant inoculation with tetanus toxin. The foreign substances were supposed to pre-engage the absorptive and digestive powers of various cells (leucocytes, etc.), so that the toxin was disposed of less readily, and more remained available for the highly susceptible nervous tissues.

The remarks just made are not intended to cast doubt on the probability that pathogenic micro-organisms produce substances which are antagonistic to phagocytosis. That the latter action really occurs has already been indicated.

Response and Adaptation by the Host.

In previous paragraphs attention was called to the fact that micro-organisms attempt to adapt themselves to their hosts, to the end that the latter

may be a more favorable medium for their existence. Thus, they increase in virulence, and disarm the host by becoming resistant to its protective agencies, and even actively antagonize the latter, particularly as regards phagocytosis, and by such means the natural immunity of the host is rendered inefficient. The host, however, is not without its own reserve forces, and, in favorable cases, after infection is once established, it responds to the presence of the micro-organisms by the production of a new supply of protective "antibodies," the effect of which is to destroy larger numbers of the invading organisms, or even to sterilize the body completely. In the latter case recovery is the probable event. This, again, may be looked on as a process of adaptation on the part of the host, in which it seeks to destroy or render less noxious the infecting microbes.

Mutual Adaptation.

In certain chronic infections it seems that a mutual adaptation takes place, so that there is a tendency for the host and parasite to live in a half-way state of harmony. Thus, in the acute stage of syphilis, the "combat" between the host and the spirochetes is an active one. Eventually, however, it would seem that the host reacts by the production of protective antibodies, a condition which results in an amelioration of the symptoms. Since the micro-organisms may remain alive and virulent in the host for a long time after this has occurred, it appears that they become habituated to the presence of the antibodies, while the host at the same time becomes habituated to the toxicity of the spirochetes. After the lapse of still further time the adaptation on the part of the patient appears to increase, and the micro-organism also

loses in virulence, if we may judge from the low infectivity usually accredited to tertiary lesions, in spite of the fact that they contain the spirochetes. This mutual adaptation, however, does not mean that the host escapes injury as a consequence of the infection. Similar conditions probably prevail in piroplasmosis and trypanosomiasis, or even in relapsing fever, as suggested previously.

Hypothesis of Welch.

What has come to be known as the hypothesis of Welch may be mentioned in this connection. 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).

Immunizing Response by Micro-organisms.

That bacteria may acquire increased resistance to the destructive agencies of the host was referred to above; but the hypothesis of Welch means a great deal more than the immunization of the bacteria against the defensive powers of the animal body. Not only may a bacterium during an infection become more resistant to the bactericidal action of the body by producing antibodies to those bactericidal agencies, or by its ability to absorb

and dispose of a greater quantity of bacteriolysin or opsonin; 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, the occurrence of which under artificial conditions was shown by Wechsberg in relation to the diphtheria bacillus; 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 to the theory; it is not at all necessary that they be toxic to 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 be 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.

In some support of this general hypothesis is the observation that the strongest leucocidin (a toxin for leucocytes) can be obtained from the staphylococcus by inoculating this organism into a serous cavity of animals, the toxin being obtained subsequently from the mixture of cocci and leucocytes.

Investigations have not advanced far toward the positive determination of the correctness of the hypothesis.

In Ehrlich's conception of "atreptic" immunity, the possibility is entertained that micro-organisms sometimes may not be able to grow in a host because the nutritive conditions are not suitable. It probably would be granted that proper nutritive substances are present in the host, but it is assumed that they may be so intimately and firmly bound to other tissue constituents that they are not available to the micro-organisms as food. Virulent micro-organisms find the nutritive substances already available, or, if this is not the condition, the injury which they effect on the tissues in the first instance results in a splitting of the constituents so that the food substance (rather a specific food substance) becomes available. Avirulent organisms not having the power to produce this splitting result do not proliferate to any great degree and soon become the prey of the protective agencies, such as the leucocytes and bacteriolysins. With this theory in mind, one recurs to the "exhaustion" theory of immunity at one time entertained by Pasteur, and cited in a previous chapter. This difference appears, however, that in Pasteur's theory an entire absence of suitable food was assumed, whereas in the atreptic theory the food substance may be present but not available for the use of the organisms. The atreptic theory is put forward as being a possible factor in resistance to some infections by some hosts, not, of course, as a theory intended to supplant other general theories of immunity.

PART TWO

CHAPTER VIII.

TYPES OF IMMUNITY.

By immunity we understand that condition in which an individual or a species of animal 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 were said to possess 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. The fact that an individual is free from gout, diabetes or any other metabolic disturbance, cannot be taken as indicating an immunity from these diseases. Inasmuch as the metabolic diseases appear to depend on the failure of certain organs to perform their functions normally, for some one or more reasons, we can only infer that in those who are free from such diseases the corresponding organs are in a state of normal activity. Similarly an individual or race which is free from an infectious disease because of lack of opportunity to contract it would not be classed as immune.

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 in-

fected 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 exists 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 contrast 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. Such immunity, however, is very transient in character, and is "passive" in its type; it depends on the transfer of protective substances from the circulation of the female parent to that of the embryo *in utero,* and some weeks after the birth of the latter these substances are eliminated and the immun-

ity disappears. That of the parent, however, persists for a much longer period; it is "active" in character, as explained later. Even in a given family, however, there are often marked variations in susceptibility and resistance. One child in a family may contract scarlet fever, while another, living under exactly the same conditions, may escape it.

Susceptibility also is often acquired in a more or less evanescent way. The resistance of an individual may vary greatly at different times and under different conditions. These are accidental, acquired states such as may be occasioned by exhaustion, hunger, exposure to cold and other unhygienic conditions.

Acquired Susceptibility.

Recently, the existence of a specific acquired susceptibility to various proteid substances and bacterial products has been determined by experimentation. An injection of serum or various proteids into the proper animal renders it extremely susceptible to a second injection; and a person who is suffering from a particular infection shows unusual sensitiveness to the products of the organism causing the infection, as shown by the tuberculin reaction of Koch. Such animals and individuals are said to have been *sensitized*. This subject will be discussed later under "Anaphylaxis."

Many of these facts 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 Immunity.

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

Although the subject is still in a developmental state, the conditions indicate that there are two factors in antibacterial immunity, one in which the plasma or serum has a marked power of killing the micro-organisms, the other in which the destruction is largely by means of phagocytic cells. The former was just referred to. Both agencies operate in many infections, as in typhoid, cholera and others; whereas in other instances (streptococci, staphylococci) phagocytosis is the only detectable protective agency. In most cases phagocytosis cannot take place until certain constituents (opsonins) in the plasma or serum have "sensitized" the bacteria. In case of recovery the power of phagocytic destruction is usually enhanced. We have, therefore, to recognize phagocytic immunity as a type, or at least as an important factor, in both natural and acquired resistance.

Phagocytosis.

In contrast to infections of the type referred to above are others in which the symptoms are produced by soluble toxins, the ectotoxins, which are secreted by the micro-organisms.

Antitoxic Immunity.

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, particularly in acquired immunity. In many examples of natural immunity, the resistance cannot be attributed so specifically to antibacterial or antitoxic serum properties. This is referred to later.

In the types of immunity referred to, the factors on which the resistance appears to depend, i. e., the germicidal or antitoxic action of the serum or the germicidal power of the leucocytes, are susceptible to experimental demonstration. We are familiar with another type of resistance, however, which finds no expression in the form of antitoxins or other antibodies. This relates par-

ticularly to habituation to various drugs, as morphin, cocain or arsenic, to which a high resistance may be acquired. The true explanation of such resistance is not known, but it would appear to depend on some acquired property by the cells which were originally more susceptible; in other words, it would appear to be an immunity which is fixed in the cells, a static immunity, so to say, if the expression may be coined. It may depend on habituation alone, on an increased power of destroying the poison by the cells, or on a decreased affinity of the cells for the poison.

A similar process may exist in acquired resistance to some of the more chronic infections, as tuberculosis, leprosy and others, although very little is known definitely concerning it.

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 indications of the occurrence of such a reaction lie, first, in the recovery of the patient, and, second, in the new antitoxic or antibacterial power which may be demonstrated in the serum or the increased phagocytic power of his leucocytes. 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 therapeutic 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 im-

mune 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 antibacterial or antitoxic, or, to a certain extent, opsonic (phagocytic).

Relative and Absolute Immunity.

Any one of the types mentioned may be either relative (partial) or absolute (complete). If the immunity is 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 the agencies which may contribute to immunity in one disease or another may be given, although we must recognize

that it probably does not include all conceivable factors.

FACTORS IN IMMUNITY.

NATURAL IMMUNITY:

1. Antibacterial properties of the serum or plasma.
2. Antibacterial properties of the leucocytes in cooperation with opsonins (preparers for phagocytosis).
3. Antitoxic properties of the serum or plasma, resulting in a simple binding or neutralization of toxins. Hypothetically, too, certain ferments of the body may split or decompose toxins.
4. Total insusceptibility of the body to endotoxins or ectotoxins. There is reason to believe also that comparative resistance sometimes depends on the fact that a toxin has a stronger affinity for organs of less vital importance for the life of the individual than it has for more important organs. The chicken, for example, is very resistant to tetanus, although its serum contains no antitoxin. It yields, however, when inoculations are made directly into the nervous tissue.
5. The "atreptic" immunity of Ehrlich, in which microorganisms do not find suitable food, or food in available form, in the body of the host. This is theoretical at present.

ACQUIRED IMMUNITY:

1. Increased antibacterial properties of the serum or plasma.
2. Increased phagocytic power of the leucocytes, and perhaps other phagocytic cells, depending on the increased formation of opsonins.
3. Increased antitoxic properties of the serum or plasma.
4. Habituation of the cells to bacterial poisons.

Under 3 and 4, we may have also to consider an increased power of splitting or digesting toxic substances. Of this, however, we know nothing definite.

One and 3 (possibly also 2) may be passive as well as active. These subjects receive further consideration in subsequent chapters.

CHAPTER IX.

NATURAL IMMUNITY.

Natural immunity to infection depends, first, on certain obstacles to invasion which are afforded by the body surfaces and the germicidal effect of their secretions; and, second, on antibacterial and antitoxic forces which are present in the cells and fluids of the interior body.

(1) *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.

Subcutaneous Connective Tissue.

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

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 re-

sistance 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, in part, at least, is due to the tortuosity of the channels, 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 toward 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. Very many 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 movement 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, as bronchitis, pneumonia, influenza, tuberculosis, are by no means clearly understood. Variations in individual resistance, here as in other parts of the body, such as may be caused by exposure to cold, 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 micro-organisms.

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 indicates. 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 (strep-

tococcus, 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 in the presence of infection enormous quantities of phagocytes can be called into action quickly.

The protective properties of the genito-urinary surfaces are not different in principle from those already mentioned (vaginal acidity, mechanical and perhaps bactericidal cleansing by the menstrual flow, urinary irrigation).

(2) *Internal Protective Agencies.*

A. Inflammation.

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

Nature of Inflammation.

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 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 char-

acteristic 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 strain. 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, 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. The phenomenon of living cells moving toward or away from certain other cells or substances is an expression of affinity and this affinity is known as 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, but some of them take up such cells as erythrocytes and other tissue cells readily. 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 held 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. This is probably true in many instances. But when we learn that after ingesting the bacteria the phagocytes 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 for many micro-organisms; 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 may contain natural

antitoxins, and, in addition, it may be of value in lessening the toxicity of poisons by diluting them, aiding in their elimination, or destroying them by means of ferments (!).

Fibrin. The abundant deposit of fibrin seen in some inflammations is of mechanical value by hemming in the infection and by 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, and Bier's passive congestion treatment, in local inflammations, finds a logical explanation in view of the facts mentioned, in that they increase congestion, which hastens the exudation of plasma and leucocytes and the proliferation of cells, and accelerate the elimination of toxic substances.

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."

B. Properties of the Serum and Plasma.

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

Natural Immunity.

The term "natural immunity," however, as indicated in the preceding 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 micro-organisms or toxins are injected directly into the tissues or circulation. At the same time every-day observation provides many examples.

Zoological Relationships.

In certain instances natural immunity or susceptibility shows a relation to zoological affinities. Thus only man and the higher apes are susceptible to syphilis; and only animals which are closely related to cattle, as sheep, goats and other ruminants, suffer from rinderpest. There are many

exceptions to this tendency, however; perhaps the most striking example is found in the fact that whereas sheep ordinarily are extremely susceptible to anthrax, Algerian sheep are relatively immune. Similarly the white rat is immune (relatively) and the wild rat is susceptible to anthrax.

Factors in Natural Immunity.

Natural immunity may depend on a lack of pathogenicity on the part of the organism for the host, which is equivalent to insusceptibility on the part of the host; or, on the presence in the host of a sufficient quantity of antibacterial and antitoxic substances; or, as suggested by Ehrlich, on the inability of the micro-organism to proliferate in the host because proper nutritive substances are not present, or, if present, are bound to other substances in such a way that they are not available as food for the organisms.

As stated in the preceding chapter, immunity may be either antibacterial or antitoxic, i. e., the immunity may in one case depend on the power of the animal's tissues and fluids to destroy the micro-organisms, or, in another, on their power to neutralize or destroy the bacterial toxins.

The distinction between antibacterial and antitoxic immunity is demonstrated more readily in acquired than in natural immunity. When one has recovered from typhoid fever, for example, his serum has acquired an increased power of killing the typhoid bacillus, while at the same time it appears to have little or no power of neutralizing the poisons of this organism. Acquired immunity to diphtheria, on the other hand, is characterized by the power of the serum to neutralize the toxin of the diphtheria bacillus, although it hardly

exceeds normal serum in its bactericidal power for the bacillus itself.

Determination of Types of Immunity.

Having ascertained by observation or experiment that a certain species has a degree of immunity to an infection, certain lines of investigation may be followed for the purpose of determining the character of the immunity. If the animal fails to become infected following the injection of a living and virulent culture, it is fair to assume that the organisms have been killed within the body of the animal. That this has been the result may, indeed, be determined by microscopic examination of the different tissues and by the inoculation of culture media. It is often desirable to determine the extent to which micro-organisms are eliminated through the excretions (urine and feces); this is best done by the culture method, but it is often a difficult technical problem.

The natural antibacterial forces with which we are familiar consist of the germicidal action of the serum and plasma, and the phagocytic and destructive action of the leucocytes, endothelial and perhaps other cells. It is difficult to obtain satisfactory results by a study of these forces within the body of the animal, particularly as regards the bactericidal action of the serum and plasma, hence such studies are usually carried on outside the body. Such experiments are open to the criticism, however, that the artificial conditions are far removed from those of the body, and that the results do not always justify us in drawing conclusions regarding the course of events within the body. Phagocytosis under natural conditions is more susceptible to study, and it is to be remembered that Metchnikoff came to his far-reaching

conclusions regarding the importance of phagocytosis purely from a study of the process *in vivo*. In spite of this we have come to a better understanding of the mechanism of phagocytosis by studies of the phenomenon under artificial conditions, as will appear later.

Germicidal Action of Serum.

In determining the germicidal action of serum, which should be freshly obtained, it may be mixed with a suspension of the microbes in a number of test-tubes, varying amounts of serum being used with constant amounts of the bacteria in the different tubes. At a subsequent period, from three to twenty-four hours later, cultures on Petri plates are made from these mixtures. The numbers of colonies which appear in these cultures, compared with 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, at least in part. 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. Many micro-organisms, indeed, which are not destroyed by serum at all, are readily ingested and killed by leucocytes (staphylococci, streptococci).

Phagocytosis in Vitro.

As a consequence of fundamental studies by Denys and Le Clef, by Leishman, by A. E. Wright and others, it is now possible to study phagocytosis *in vitro* with a degree of accuracy not to be approximated in the living body. It has been learned that leucocytes, as a rule, are not able to take up bacteria until the latter have been acted on (sensitized) by some substance which is contained in the serum. Wright gave the name of "opsonins" to these substances. Some of them are susceptible to heat (55 C.), while others are much more resistant. To the latter, which are greatly increased by infection or artificial immunization, Neufeld has applied the term "bacteriotropins."

Leishman's method is to mix a suspension of the bacteria with defibrinated blood (containing leucocytes), incubate the mixture for fifteen or more minutes, to make and stain spread preparations on slides, and then count the number of bacteria which have been ingested by the leucocytes. The average number taken up by fifty or more leucocytes constitutes the "phagocytic index." Wright has varied this technic in order to study the opsonins quantitatively, as will be described later.

Experiments have shown that all normal serums contain opsonins for a variety of micro-organisms, and all micro-organisms are susceptible to phagocytosis by the blood of one or more animals, in case their virulence is not excessive. We have every reason, therefore, to believe that phagocytosis is

an important natural protective factor, and in some instances it is the only one which can be actually demonstrated, as in the case of staphylococci and streptococci.

That organisms are often destroyed after being ingested by the leucocytes is manifest from changes in form which they undergo, and from the loss of their staining power. Cultivation experiments have also shown that the leucocytes are able to kill certain bacteria. In such experiments, the technic which was mentioned in testing the bactericidal power of serum may be used, in this case, however, substituting defibrinated blood, which contains leucocytes, in place of the serum. If the bactericidal power of the defibrinated blood is greater than that of the serum alone, the effect of the leucocytes becomes apparent.

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 at 55 C. for thirty minutes. As will be indicated later, the "alexins" are more complex than Buchner supposed.

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, or may not, contain antitoxin. 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. On the other hand, if the serum shows no such antitoxic effect, we must conclude that the resistance of the animal is due to other causes; as, for example, non-susceptibility of the tissues, power of the living cells or ferments to destroy the toxin, or absorption of the toxin by tissues of secondary importance to life.

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, or dependent on non-susceptibility. 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, or the micro-organism cannot be cultivated, as in scarlet fever, measles, etc., that is, if the virus and its toxin cannot be obtained in quantities, the nature of the resistance is not at present open to determination.

It is seldom that natural resistance is absolute. Pasteur found that the great immunity of the chicken for anthrax could be overcome by im-

mersing the animal in cold water, the reduction in body temperature supposedly decreasing the resistance. It was stated previously that physical exhaustion, hunger and exposure to cold may also reduce natural resistance. Pestilence and famine often go hand in hand.

Relative immunity.

Similarly, immunity to toxins usually is relative. As an illustration of natural immunity to toxins, the following table serves a good purpose. The horse is the animal of greatest susceptibility to tetanus toxin. If the minimum fatal amount of 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 fata
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 or 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 satisfactory 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. Non-susceptibility 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 to tetanus which the rabbit has over the guinea-pig is due largely to the fact that the rab-

bit'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. We may, then, enumerate the following as the factors which probably are responsible for the different grades of natural immunity and susceptibility to various bacteria and their toxins: the bactericidal and antitoxic powers of the serum and plasma; the destructive effects of the phagocytes and other cells on both bacteria and toxins; a possible absolute non-susceptibility in some cases (the absolute non-existence of suitable cell receptors); the lack of suitable available food for the microorganisms in some instances (atreptic immunity; see the preceding chapter); the overwhelming distribution of the "suitable" cell receptors in organs of less vital necessity for the individual, thus diverting the poisons from the more important organs.

This knowledge is very general, however, and in many specific instances we continue to be in doubt regarding the exact conditions which are responsible for natural immunity and susceptibility. We have no reason to believe that any one factor is operative for all infections, although phagocytosis appears to be more general in its action than the other processes mentioned. Each disease must be studied as a unit in relation to each species of animal. In one instance the resistance or susceptibility may depend on the bactericidal power of the body fluids; in another, on the germicidal action of the leucocytes and other cells; in another, on the antitoxic (destructive) action of the cells or ferments with or without the presence of true "anti-

toxins" in the serum, etc. There is reason to believe that two or more different protective processes may come into operation at the same time against a given infection.

Regarding natural antitoxic immunity, it seems probable that we have no example in which the resistance can be satisfactorily explained solely by the quantity of "antitoxins" which are demonstrable in the serum; rather we must assume the existence of other means of destroying and resisting toxins, as mentioned above.

In order that a pathogenic organism may 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.

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 agglutinated and destroyed the red blood cells of the injected animal. The process, 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, nephrotoxins 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 many instances, a foreign serum which is not particularly toxic on first injection, becomes very poisonous when administered (subcutaneously) a second time. (See "Anaphylaxis.")

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 X.

ACQUIRED IMMUNITY.

Acquired immunity may be either active or passive: active when it arises as a consequence of infection or artificial immunization (vaccination); passive, when protective or curative serums are injected.

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 specific in character: that is, 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. The mere fact of recovery, however, is sufficient evidence of at least a temporary immunity. It is evident, therefore, that among the various infectious diseases different grades of active immunity must be recognized.

Certain chronic diseases are of particular interest in this connection, as pointed out in Chapter

Chronic Diseases.

VII. On first thought it would seem that immunity can have no place in an infection of long duration, from which recovery is rare or does not occur. This, however, is not necessarily true, and the very chronicity of the infection may in some instances depend on the establishment of a certain degree of acquired immunity. It is, of course, possible that in other instances chronicity depends on a low degree of virulence on the part of the micro-organisms or a low natural susceptibility on the part of the host. Sleeping sickness may be taken as an example of a chronic disease, the prolonged course of which, in all probability, depends on the formation of protective substances. This is indicated from the fact that an acute is followed by a chronic stage. At the height of the acute stage trypanosomes are very numerous in the blood, but after a time their number decreases and eventually it is difficult to find them except in organs which serve as reservoirs for them. Ehrlich speaks of this type of immunity as *Immunitas non sterilisans.* It may disappear more or less completely, its disappearance being marked by a recurrence of acute trypanosomiasis.

The existence of this temporary immunity to trypanosomiasis was demonstrated in mice by Franke. When mice, infected with mal de caderas (a variety of trypanosomiasis) are given an injection of a sufficient quantity of "trypanrot," all the trypanosomes are killed and the cure is immediate. If a smaller quantity of "trypanrot" is injected, it may still be sufficient to free the circulation from parasites for twenty or thirty days, after which general invasion again occurs. During this period of comparative freedom from parasites the animals

are relatively immune, which is shown by inability to reinfect them with trypanosomes of the same species. After this period is passed they succumb very quickly. That the heightened resistance is not due to the presence of a residuum of "trypanrot" in the body is shown by the fact that susceptibility for other species of trypanosomes (as nagana) is retained. In other words, this temporary immunity is somewhat, if not absolutely, specific. It probably is brought about by rapid active immunization consequent on the disintegration of many parasites following the administration of the "trypanrot."

The conditions in syphilis and piroplasmosis would seem to be similar to that in sleeping sickness; i. e., during and following general invasion, reinfection from without does not occur, although the disease is still active in some part of the body. In both syphilis and trypanosomiasis reinvasion from within may occur, presumably following the disappearance of the temporary immunity.

Periodic Immunity.

In still another infection, relapsing fever, it would seem to be similar to that in sleeping sick- and reinvasion alternate before the course is completed. It is not clear why the micro-organisms are not entirely killed off during the periods of temporary immunity. Several factors may be involved. During the periods of remission the spirilla leave the general circulation and are found in some of the solid organs, particularly the spleen. At this time they may be protected to some degree by an existence in organs which are relatively free from germicidal agents. During this period also the less resistant organisms may be destroyed and those which remain may undergo an adaptation to

the protective agencies of the host, which would be equivalent to an immunization against the antibodies of the host. The recurrence of general invasion may also coincide with a disappearance of the general blood immunity.

Protozoan Bacterial Infections.

Concerning such chronic infections Ehrlich may be quoted ("Chemotherapeutische Trypanosomen-Studien," *Berl. klin. Wchnschr.*, 1907, No. 9-12): "In accordance with the views which Robert Koch has developed regarding malaria, I assume that in various protozoan diseases an immunity of permanent character is far from occurring as readily as in the majority of the bacterial diseases, and that a certain degree of permanent immunity, characterized by the presence of antibodies, is obtained only after prolonged invasion of the body, demanding particularly a large number of recurrences. If the immunity attained is not sufficient to destroy all the parasites, those which remain accommodate themselves to the injurious agents which are present."

It has, indeed, been suggested that a general principle prevails to the effect that any infection in which an attack confers strong and lasting immunity must be bacterial rather than protozoan in its etiology. This does not imply, of course, that all bacterial diseases confer strong immunity; there are many examples to the contrary, as already stated, although a sufficient number of examples are known to render it of suggestive value in the study of diseases of unknown etiology.

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 throughout. Epidemics of benign smallpox occur frequently. In these instances it seems probable that the mild character of the disease depends on the low virulence of the strain which causes the infection; 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 so to 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 called variola inoculata. 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. 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 was referred to in Chapter VII under "passage."

Attenuation. 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 virus is 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. This point, however, is difficult to determine in relation to diseases of unknown etiology, or in the event that the micro-organism does not lend itself to the necessary experimental manipulations (smallpox, hydrophobia). It is possible that the protection may be largely antitoxic in some instances. In Wright's method of the therapeutic

inoculation of killed cultures or bacterial products (e. g., staphylococci, tuberculin) the attempt is definitely made to increase the opsonins and other antibodies in the patient's blood.

The Serum in Active 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. On the other hand, acquired immunity is not always represented by an increase in the bactericidal or antitoxic power of the serum. Bactericidal antibodies may, indeed, be formed, but, if so, the micro-organisms concerned are not susceptible to their action.[1] This is the case with the

1. By the method of complement fixation, which will be explained later, it has indeed been shown that practically all organisms are able to cause the formation of some type of antibody.

streptococcus, staphylococcus, pneumococcus, and several others.

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, and there is now no lack of evidence to show that they are of great importance for acquired immunity, at least in many instances. Particularly, Metchnikoff and his followers have provided us with many observations which go to prove this point.

These investigators showed 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 produced these substances in excess and excreted them into the plasma.

Opsonins.

The views of Metchnikoff regarding the importance of phagocytosis have been greatly strengthened in recent years as a consequence of quantitative studies of phagocytosis *in vitro*. As already stated, phagocytosis of bacteria depends on their first being "sensitized" by the opsonins which are present in the serum. In 1895, Denys and Le Clef demonstrated that the serum of animals which had been immunized with streptococci induced a much greater phagocytosis and destruction of these organisms than normal serum, determining their results by means of plate cultures and microscopic studies. More recently, by means

of the technic evolved by Leishman and by Wright, this principle has been found to have a wide, almost universal application: in other words, active immunization, as in a natural infection or by the injection of bacterial cells, is almost invariably accompanied by an increase in opsonins, which appears to coincide with an increase in the phagocytic power of the blood.

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 absorp-

tion and destruction of toxins in the case of antitoxic immunity.

"Stimulins" and Opsonins.

It is now known, as stated, that an immune serum favors phagocytosis because of its action on the bacteria rather than on the leucocytes; hence the position of Metchnikoff's "stimulins," which were supposed to stimulate the leucocytes to an increased phagocytosis, does not seem to be on a good footing at present. The value of the opsonins in passive immunity is, indeed, an unknown factor; the question is hardly determined finally. Some of the opsonins deteriorate very quickly; hence they could be of no value in serums as they are placed on the market. Others are more resistant, and may have a certain value in passive immunization, although they probably do not approximate in importance the bactericidal and antitoxic substances.

Summary.

By way of summary we may say that bactericidal substances, antitoxins and opsonins are the known and demonstrable factors in active immunity. It does not follow that all three factors come into play in every conceivable infection; or that if they do, in some particular disease, the three are equally important. Thus, in typhoid fever, the serum has an enhanced bactericidal power, and investigations seem to show that the opsonins are also increased; on the other hand, we have no evidence to show that acquired immunity to typhoid fever is antitoxic. In diphtheria and tetanus, the immunity is represented by the presence of antitoxins in the serum, whereas the opsonins and bacteriolysins appear to be of less importance.

Another condition is found in infections with staphylococci, streptococci, pneumococci and some

other organisms, in which the only demonstrable change of importance is an increase in the opsonins, and with this an increase in the power of phagocytic destruction of the cocci.

Habituation.

In some chronic infections it is possible that the individual shows a resistance to the bacterial toxins, which is on the order of habituation, or adaptation, and which is not represented by any demonstrable antitoxins. Thus, by the use of gradually increasing doses of tuberculin, an individual may eventually tolerate large doses which in the beginning would have been very toxic.

In spite of this acquired resistance, however, the body appears to form no true antitoxin for the tuberculin.[2] After the cessation of treatment the resistance of the individual gradually returns to normal; that is to say, the cells return to their original susceptibility.

The possible relation of anaphylaxis to acquired immunity will be discussed in the chapter on "Anaphylaxis."

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

2. Such a course of treatment does cause the formation of antibodies of a specific character, but they appear not to be antitoxic in character. The "antituberculin" which Wassermann recognizes by means of the method of fixation of complement has not been shown to be an antitoxin.

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.

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 has been supposed that in 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 asserted 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-proteidins," on which acquired immunity depends.

It is also stated that with "pyocyanase-immune-proteidin" it is possible so to immunize a rabbit that a subsequent (twelve 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.

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 the 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 (alexin) to the peritoneal cavity. The suggestion 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 cited.

Immune Cytotoxins.

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

Immune Agglutinins.

By systematically injecting an animal with a bacterium or with any tissue cell, agglutinating substances (agglutinins) are formed and may be demonstrated in the serum. Like other antibodies, they are highly specific for the cell used in the immunization.

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.

mmune 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, particularly as regards the differentiation of species.

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 "Precipitins.")

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, a great many substances for which antibodies can not be obtained; this is true for substances of known chemical composition, such as acids, bases, salts, and for the alkaloids (strychnin, morphin, aconite, etc.)

CHAPTER XI

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. According to his original conception they have the following properties:

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 biologic 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]

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

The incubation period may be shortened experimentally by the injection of large quantities of toxin, but it can not be eliminated entirely. The poisons of snake venoms appear to act without incubation period, but they are still to be classed with the toxins, because of their power to cause the formation of antitoxins.

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 the soluble toxins of bacteria is relatively simple. It is necessary only to inoculate a suitable fluid medium with a culture of the microorganism, 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 botulinus,* 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 particu-

lar sense specific toxins. Some micro-organisms, however, produce more than one toxin. The tetanus 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 hematoxin.

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 infections with streptococci and staphylococci, for example, in which 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 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 MacFadyen Method.

MacFadyen, by grinding large masses of typhoid bacilli and other organisms which had been rendered brittle by the temperature of liquid air, obtains from these organisms a toxic cell juice. The efficiency of the antitoxins which he is said to obtain by such immunization has not been demonstrated practically. It seems improbable that immunization with such "toxins" 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 optimum hygienic condition, and from which rats in particular are rigorously excluded.[2] The horses

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.

are carefully groomed and nourished and given such exercise as will keep them in a healthy condition.

The toxins, in solution, are injected subcutaneously.[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) or, as is often done at present, by partially neutralizing the toxin with antitoxin. 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

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

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 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

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.

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 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 from 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.)

Standard Antitoxins.

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 director, is a dried and powdered serum of such strength that 1 gram contains 1,700 immunity units; *i. e.*, 1/1700 gm. would protect a guinea-pig against 100 fatal doses of a diphtheria toxin.[5]

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

Any other high-grade serum would have answered equally well.

The institute keeps in stock a large number of vials, each containing 2 grains 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

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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.

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

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 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.

In accordance with the Act approved July 1, 1902, the United States Public Health and Marine-Hospital Service has established a standard unit for this country. The unit is based on that of Ehrlich just described and was made by comparison with the normal unit obtained from Ehrlich's Institute, Frankfort a. M., Germany.

Antitoxins are purchased on the open markets by officers of the Public Health and Marine-Hospital Service and tested in the Hygienic Laboratory for potency, freedom from contamination by bacteria and chemical poisons, especially tetanus toxin, and finally to insure against excess of preservatives.

The method of determination of potency is similar to that used by Ehrlich and previously described.

White mice are inoculated to test for an excess of preservative. (Trikresol being the one most employed.) If the mouse shows trembling or other symptoms of poisoning after subcutaneous injec-

tion of 1 c.c. of serum, over 0.5 per cent. may be suspected.

Other toxins and bacteria are discovered by intraperitoneal injection of guinea-pig.

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.

In some cases it is desirable to concentrate the antitoxic serum.

Gibson has devised a means for this depending on the fact that the antitoxin is closely associated with or comprises the globulin of the serum. The method is as follows:

To from 10 to 15 liters of serum, a saturated solution of ammonium sulphate is added gradually until precipitation is complete. This filtrate is then removed by filtration through paper and dissolved in 12 liters of water. It is then strained through gauze to remove the filter paper. The solution is reprecipitated with ammonium sulphate and the precipitate removed as before. The precipitate is then dissolved in 24 liters of a saturated solution of sodium chlorid and filtered through gauze. This solution is allowed to stand over night and the supernatant fluid removed from any precipitate which forms. The precipitate is washed with saturated sodium chlorid and the washing added to the first solution.

The combined solutions are again precipitated with saturated ammonium sulphate solution and

the precipitate collected on filter paper and the moisture pressed partially out with filter paper.

The precipitate is then dialized in parchment paper in running water for three days, a small amount of chloroform being added as a preservative.

One-half of one per cent. sodium chlorid is then added and the solution filtered twice through Berkefeld filters.

In a similar way the U. S. Government has provided for the establishment of a legal tetanus antitoxin unit and the control of serum production.

Owing to the fact that tetanus toxin is very stable, the toxin itself is kept for comparison instead of the antitoxin as in diphtheria.

"The immunity unit for measuring the strength of tetanus antitoxin shall be ten times the least quantity of antitetanus serum necessary to save the life of a 350 gram guinea-pig for ninety-six hours against the official test dose of a standard toxin furnished by the Hygienic Laboratory of the Public Health and Marine-Hospital Service.[7]"

That the United States government is attempting to guard the quality of antitoxins on sale in our markets is apparent from the following statement:[8]

"EXAMINATION OF SERUMS MADE BY LICENSED MANUFACTURERS.[8]

"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 pur-

7. Bulletin No. 43, Hygienic Laboratory.

8. 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.

poses,' 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 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 XII.

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 must be assumed 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. Wassermann 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 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 haptophorous group, through which it unites with antitoxin in one instance or, in another instance, with tissue cells in the production of disease, but also certain constituents in which the specific activity of the substance resides, and by which it produces changes in tissue cells, on combining with them. This functional or pathogenic activity resides in this so-called toxophorous group of the molecule. Hence the haptophorous and tovophorous groups are the two structural elements of a toxin which may be recognized by biologic experiments.

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 information 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.

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 in order to distinguish it from that other constituent of diphtheria bouillon, the toxin, which causes the acute phenomena of diphtheria. **Toxon.**

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. Bordet believes that toxon and the various other constituents of toxin described by Ehrlich as separate substances, are the result of combinations of varying proportions of toxin and antitoxin. He produced comparable phenomena by mixtures of complement and anticomplement in varying proportions and noting the degree of hemolysis produced on sensitized corpuscles. In spite of the reasonableness of this theory, Ehr-

lich and his followers continue to uphold the toxon as an independent toxic substance, and have published additional experiments to support their position.

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 binding 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 proto-

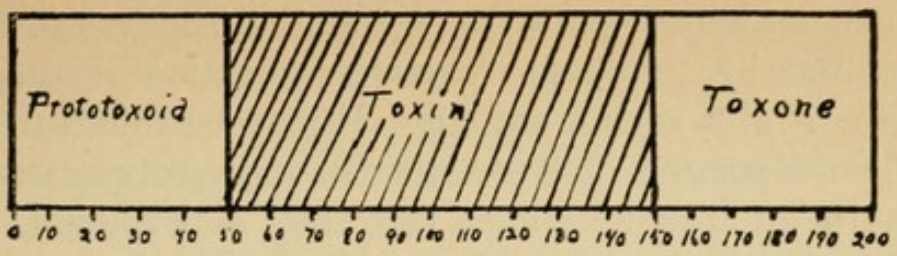


Figure 1.

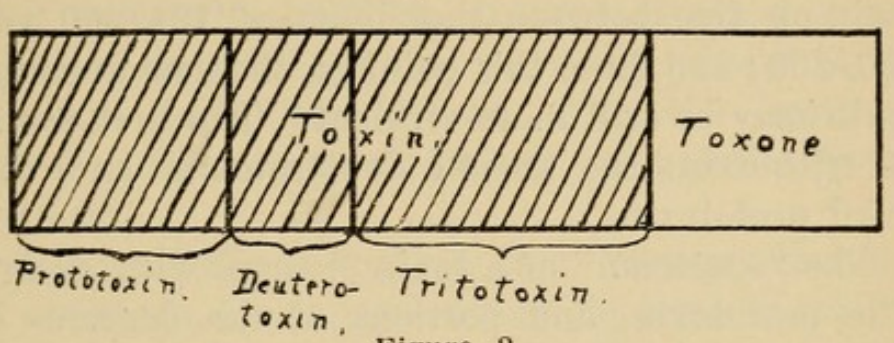


Figure 2.

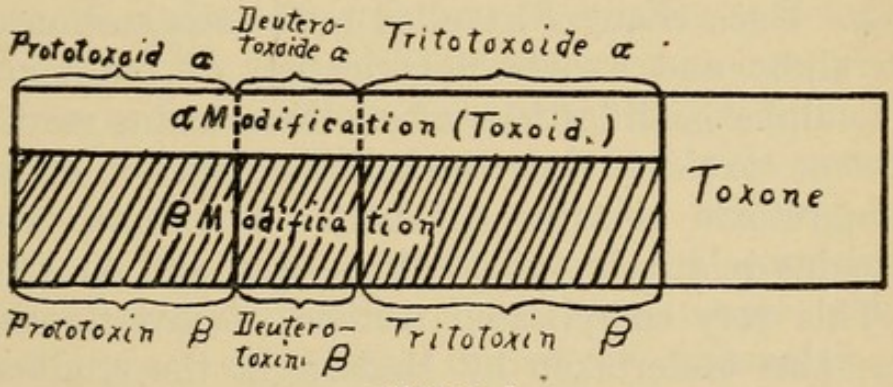


Figure 3.

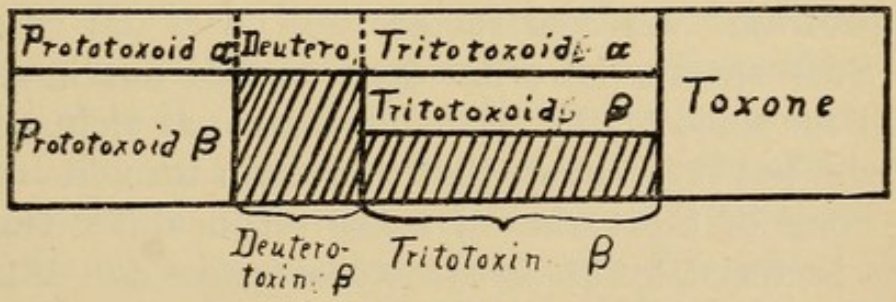


Figure 4.

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.

toxin, 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.

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.

Some of the toxins of snake venom differ from the bacterial toxins in structure (pages 428-431).

The Formation of Antitoxin.

The idea was originally advanced that antitoxin is 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 equalled 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."

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

Receptors.

Multiplicity of Receptors.

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

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 constituents, 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 num-

ber 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.

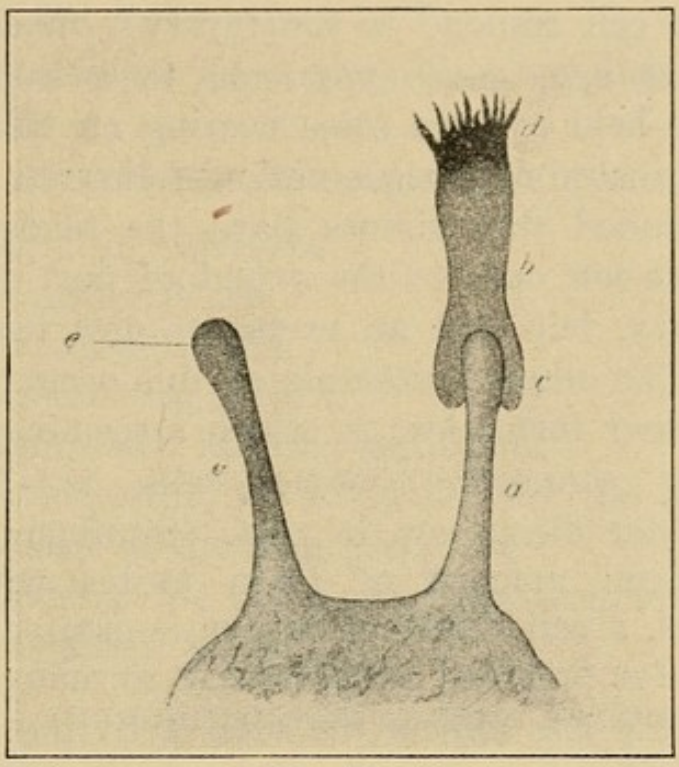


Fig. 5.—Graphic representation of receptors of the first order and of toxin uniting with the cell receptor. *a*, Cell receptor; *b*, toxin molecule; *c*, haptophore of toxin molecule; *d*, toxophore of toxin molecule; *e*, haptophore of the cell receptor. From Ehrlich's "Schlussbetrachtungen," Nothnagel's 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.

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.

Receptors of the First Order.

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.

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 for 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 (Noguchi), and also the hemolytic action of cobra venom and cobra-lecithid. (See Chap. XVI). On the other hand, it does not affect two other hemolytic toxins, staphylolysin, which is derived from the staphylococcus, and arachuolysin which is obtained from spiders (Kyes), the action of cholesterin, therefore, is in no sense specific and apparently is of a different type from that of serum antitoxins. This is further indicated by the fact that chloesterin also inhibits the hemolytic activity of certain substances which can not be classed with the toxins, *e. g.* saponin, agaricin (Noguchi). Fluids which contain cholesterin naturally, as milk, serum and bile, have a similar inhibiting power.

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 XIII.

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 Grunbaum.

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 Agglutinins.

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. Becht and Greer find that agglutinins occur in the various body fluids in the following order of concentration: blood serum, thoracic lymph, neck lymph, traces in the pericardial fluid, least and not constantly in the cerebrospinal fluid and aqueous humor. 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, according to very convincing experiments of Hektoen and Carlson, the tissue cells of the body.

Significance of Agglutination. One of the most interesting and important phenomena in the study of immunity is the so-called Pfeiffer phenomenon. 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 pour-

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.

ing 5 or 10 c.c. 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 or natural bacilli were agglutinated before they were injected 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 10 c.c. 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 from 0.5 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 XIV).

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

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.

slide. More accurate dilutions can be made by means of capillary tubes.

A convenient amount of serum is allowed to enter the tube by capillary attraction. The length of this column is marked on the tube and then successive volumes of the diluent drawn in, each being separated from the succeeding one by a small bubble of air. 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.

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 if previously heated, 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 (autoagglutinins), but often does, however, 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 XIV.

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 agglutinogen 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.

Flagellar and Somatic Agglutinogens.

Agglutinogens are said to pass through semi-permeable membranes, while agglutinins do not.

Smith and Reagh distinguish two kinds of agglutinogen in those bacteria which possess flagella, 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 ex-

posure 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

Zymotoxic Group.

Agglutinoids.

with its binding power for agglutinogen practically 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 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.

Proagglutinoids.

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 pro-agglutinoids becomes less, and at a time when they are so dilute that they have no influence on the reaction, the agglutinins

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-Agglutinin.

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

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.

Dilutions. 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 microorganism.

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

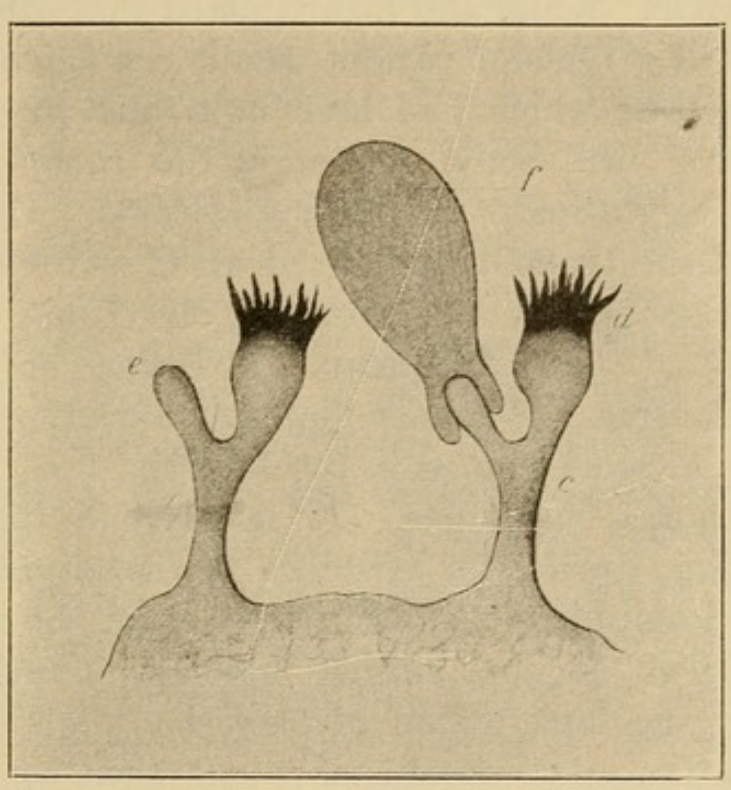


Fig. 6.—Graphic representation of receptors of the second order and of some substance uniting with one of them. *c*, cell receptor of the second order; *d*, toxophore or zymophorous group of the receptor; *e*, haptophore of the receptor; *f*, food substance or product of bacterial disintegration uniting 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.

Agglutinability 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 physico-chemical nature takes place between the agglutinin of the serum and the agglutinogen of the micro-organisms, 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. The physical nature of the reaction is strongly supported by the facts that bacteria may also be agglutinated and precipitated by well-known chemical substances, such as hydrochloric acid, and by various organic and inorganic colloids (colloidal solutions of calcium chlorid ($CaCl_2$), zinc sulphate ($ZuSo_4$), ferric hydroxid ($Fe(OH)_3$), aluminum hydroxid ($Al(OH)_3$), ferric chlorid ($FeCl_3$) and aluminum chlorid ($AlCl_3$). Some of these substances behave like the agglutinating serums in the possession of the so-called prozone; i. e., they may fail to agglutinate in more concentrated solutions, whereas after dilution, their agglutinating power becomes manifest (hydrochloric acid and the staphylococcus, according to Buxton and Rahe). Still further indirect evidence of this nature of the reaction is found in the observation, made first by Neisser and Friedberger, that two colloids which

bear opposite electrical charges result in sedimentation when they are mixed in suitable proportions. Eosin and Bismarck brown, mastic and colloidal ferric hydroxid ($Fe(OH)_3$), colloidal silicic acid and colloidal ferric hydroxid are mixtures which behave in this way. Here also the inhibiting "prozone" is obtained in concentrated solutions.

An analogy appears to exist between bacteria which have absorbed agglutinin and certain colloids in that both may be agglutinated by the addition of suitable electrolytes. This phase of the agglutination of bacteria was referred to previously. In a like manner "agglutinin-bacteria" and the colloids mentioned may alike be precipitated by various salts (electrolytes), such as the chlorids of sodium, calcium and potassium, and many others.[1]

1. Buxton and Rahe: Jour. Med. Research, 1909, xx, 113.

CHAPTER XV.

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-white 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 less likely to form antibodies for the tissue constituents of other members of the same species than for those of other species; these, when formed, are called iso-antibodies. Schutze immunized thirty-two rabbits with serum from the rabbit and obtained an iso-precipitin from only two of the number. In the third place, animals do not readily form anti-bodies 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. That bacterial precipitins are not identical with agglutinins for the same bacteria is shown by the following facts: Immunization with certain bacteria may produce agglutinin but no precipitins. Precipitins develop more slowly than agglutinins. As a rule precipitins are inactuated at lower temperatures than agglutinins.

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 lat-

ter 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.

Antiprecipitins.

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

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. Both agglutinin and agglutinogen are present in the precipitate, but there seems to be no law governing the amounts of each in the combination.

The supernatant fluid contains a remaining soluble part of both substances as can be shown by adding fresh precipitin and *vice versa*.

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 adven-

titious 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 details 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.

Colloids and the Reactions of Immunity.

(For the relation of precipitins to anaphylaxis see chapter on anaphylaxis.)

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 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

Properties of Colloids.

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 XVI.

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, as antityphoid, do kill bacteria without dissolving them, while others, as anticholera, have the dissolving power, 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 are indicated on page 254. 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 obtained with the organism of cholera. A guinea-pig is immunized against this micro-organism by injections of the killed or living bacteria. 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-shaped 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 inter-

vention 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 is not necessary to render it active for bactericidal experiments *in vitro*. It is 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 is 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. 256). 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.

Bactericidal serums are not obtained with equal readiness for all micro-organisms. We are most familiar with those which are yielded by immunization 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. This will be referred to again in relation to phagocytosis (Chapter XVIII).

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 formation 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 micro-organisms 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. (See origin of agglutinins, Chapter XIII.)

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 degenerated, its reactivation is accomplished through the complement which is present in the body of the experiment animal. There are reasons for believing 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; this will be referred to again.

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 from 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.

B. HEMOLYSINS.

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 complement 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 of 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.

Cytophilous Haptophore of the Amboceptor.

The question as to whether the corpuscles in taking up amboceptors do so by chemical combination 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 from 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 experiments 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* (complementoid obstruction) 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 re-

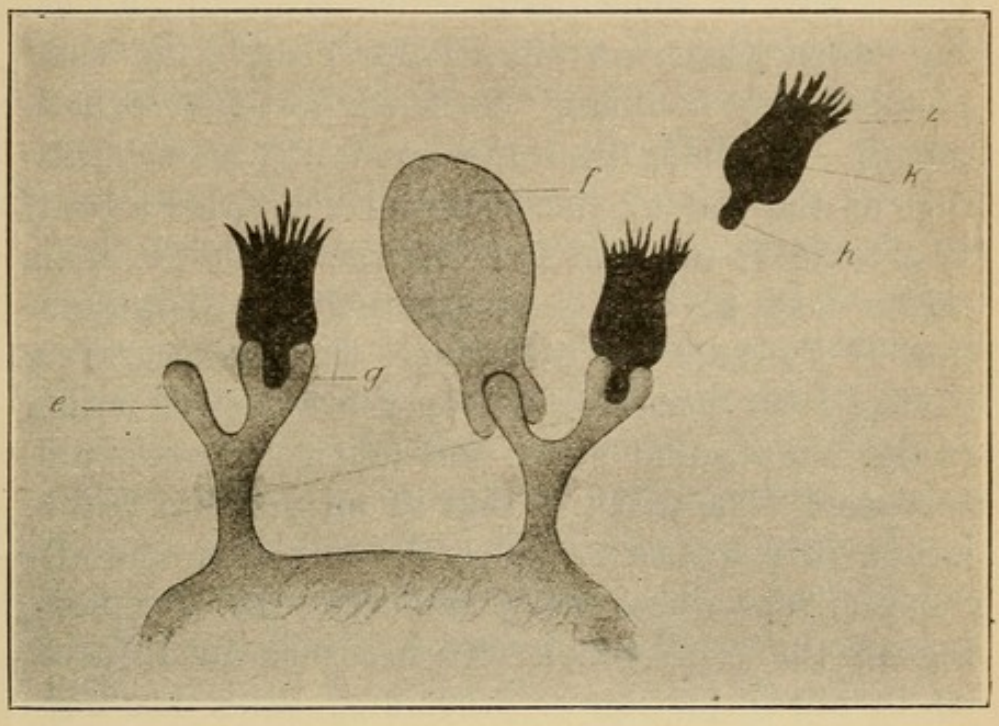


Fig. 7.—Graphic representation of receptors of the third order, and of some substance uniting with one of them. *c*, Cell receptor the third order, an amboceptor; *e*, one of the haptophores of the amboceptor, with which some food substance or product of bacterial disintegration, *f*, may unite; *g*, 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.

ceptors 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 stimulation 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 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 newformed 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 cholera, 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 (p. 280). 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 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.

Antiamboceptor.

If one immunizes with an immune serum the product is spoken of in a general way as an anti-

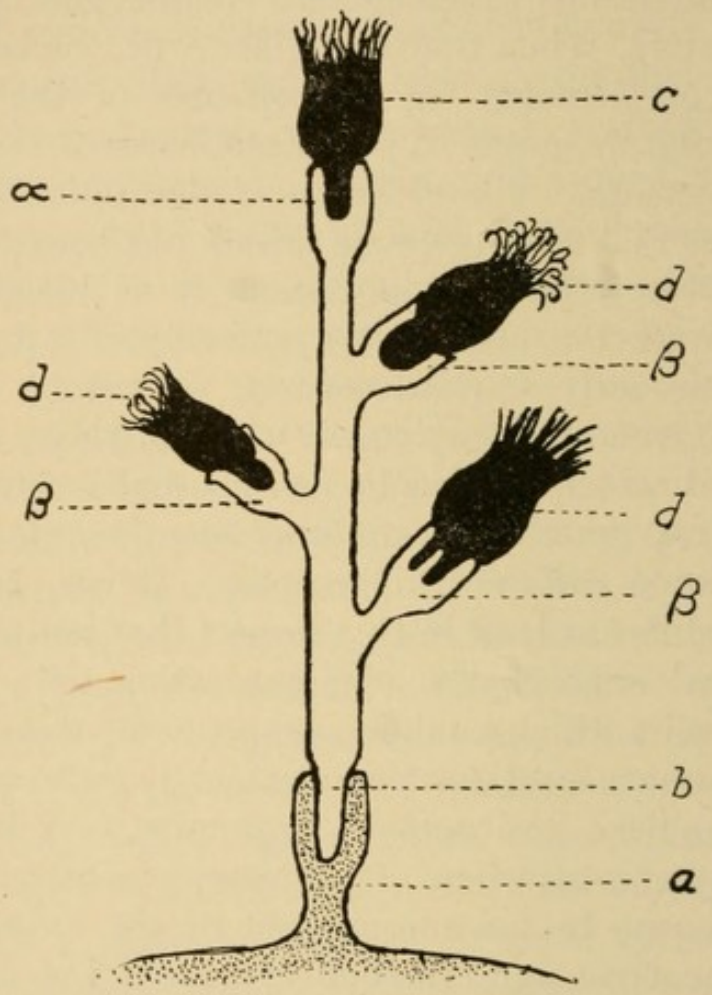


Fig. 8.—Illustrating the amboceptor with more than one complementophilous haptophore (a polyceptor). *a*, Cell receptor; *b*, cytophilous haptophore of the amboceptor; *c*, the dominant complement; *d*, the non-dominant complements; , the heptophore of the amboceptor for the dominant complement; *β*,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

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 com-

Danger of Formation of Antiamboceptor.

plementophilous 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.

Deviation of Complement and its Theoretical Danger.

A phenomenon equally of theoretical and practical importance has to do with the so-called deviation (*Ablenkung*) of complement. It has been found that the action of a bactericidal or hemolytic

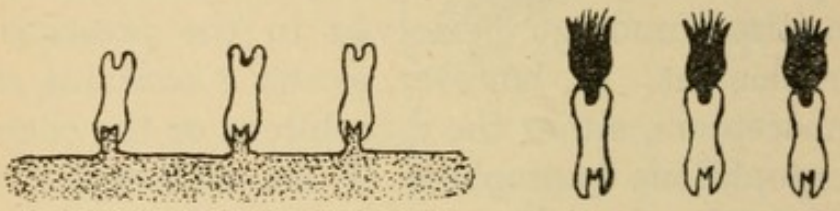


Fig. 9.—Illustrating deviation of complement. The free amboceptors have combined with the available complement, and thereby prevented the latter from activating the amboceptors which have united with the bacterial cell. (From Neisser and Wechsberg.)

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 direction of activity (Fig. 9). This amounts to a protec-

tion 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 venoms of many serpents have the 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 contains two well-known toxins, one for the nervous tissue and one which dissolves erythrocytes, the neurotoxin having the greater pathogenic significance. Cobra venom also agglutinates red blood corpuscles, and Flexner and Noguchi found that it contains special toxins for the cells of various organs (cytotoxins). The venom of the rattlesnake, on the other hand, is neurotoxic to a less degree, but has a pronounced influence in causing capillary 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 neces-

sary 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 Activation

In attempting to discover the nature of the complement which is present in the erythrocytes, various substances existing normally in the red cells, as cholesterin 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.

According to Bang lecithin itself has no activating power for snake venoms. He attributes the hemolytic action of the product of the action of lecithin on venom according to the Kyes technic as due to pre-existant impurities in the lecithin That hemolytic substances exist in unpurified lecithin there is no doubt. The lecithin used by Kyes in his experiments, however, was prepared with great precaution to avoid the presence of such substances.

v. Dungern and Coca attribute the action of lecithin or venom to a splitting off of hemolytic products from the lecithin and fail to obtain an "antilecithid" by immunization. They also point to a similarity between Kyes elementary analysis of the lecithid and that of lecithin and confirm his analysis. Their view is supported by Manwaring.

Kyes, however, had pointed out the fact that elementary analysis is, as a general rule, insufficient to determine differences in substances concerned in immune reactions and by a determination of molecular weights shows that cobra lecithid

has a much larger molecule than lecithin itself. His immunization of animals to produce an anti-lecithid extended over a much larger period than those of v. Dungern and Coea.

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 Complement by Salt.

Another 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 sub-

ject 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.

Further Analysis of Complement.

The work of Ferrata, Hans Sachs and Teruuchi, Brand and Hecker has shown that complement may be divided into two parts by the separation of the albumin and globulin contents of the serum containing it.

Neither of these two parts alone has the power to bring about hemolysis in corpuscles previously sensitized by the addition of amboceptor. When combined, however, hemolysis takes place as before separation.

Absorption of the globulin fraction takes place when it is added to sensitized corpuscles, resulting in "Persensitized corpuscles" (Michaelis and Skwirsky), which then undergo hemolysis on addition of the albumin fraction. The albumin fraction, on the contrary, is incapable of being bound by the sensitized corpuscles in the absence of the globulin fraction. Brand therefore terms the globulin fraction as complement "middlepiece"

and designates the albumin fraction as complement "end-piece."

The end-piece, middle-piece and amboceptor bear the same relation to each other as whole complement amboceptor and antigen.

The middle-piece is thermostabile when attached to the amboceptor antigen complex, but thermolabile when heated alone or in combination with the end-piece. The end-piece is thermolabile. In specific complement deviation, it is the middle-piece which becomes bound, the end-piece remaining free.

Attempts to ascertain the nature of these two parts by substitution of lecithin and other lipoids have so far been without result in explaining the nature of the two components (Liefman and Cohn).

CHAPTER XVII.

COMPLEMENT DEVIATION.

In 1901, Bordet and Gengou observed that when an antigen was mixed with its specific antibody in the presence of complement, the complement was fixed or bound and thus rendered unavailable for further reactions. This phenomenon has since become widely known as complement deviation, complement binding, or complement fixation; and the principle underlying it has become of extreme value in the determination of the presence of substances whose interaction is followed by no readily perceptible result such as lysis or precipitation.

It will be seen that such a reaction is analogous to certain chemical reactions such as the combination of ordinary acids and alkalies in which the presence of the reaction is determined by the use of indicators such as litmus or phenolphthalein. Bordet and Gengou used as an indicator a hemolytic system of erythrocytes with their specific amboceptor. In this way, for instance, by mixing typhoid bacilli with antityphoid serum, incubating for a time and then adding erythrocytes sensitized with their inactivated antiserum, it was observed that hemolysis did not take place. The complement in the antityphoid serum had been fixed to the typhoid bacilli by the typhoid antibody and was thus rendered unavailable for the hemolysis of the sensitized erythrocytes, subsequently added. Bordet also showed that other indicators such as

a bacteriolytic system could be used instead of hemolysis, in order to test for the binding of complement.

Non-Specific Complement Inhibition.

Complement deviation obviously belongs to a large group of complement inhibition phenomena and since some of these have a very close bearing on the complement deviation by means of antigen-antibody complex it is well to review them.

Ehrlich and Morgenroth made use of the complement inhibition of lowered temperature to separate complement from amboceptor.

Hektoen and Ruediger found that various ions might render complement inactive. Certain substances which in themselves are hemolytic have been shown to antagonize complement action. Among these are to be mentioned bile salts, salts of fatty acids, lecithin cholesterin and other lipoid bodies.

Suspensions of finely divided substances have been demonstrated to inhibit complement action and the assumption that their ability to antagonize complement is due to their adsorptive property is highly probable. Kaolin, chalk, carbon, sand, etc., have been used in this way.

A wide variety of colloidal substances have been shown to inhibit complement; examples of these are gelatin, peptone, aleuronat, albumoses, etc. Lastly, extracts of bacteria, normal and pathologic tissue extracts and the body juices work as complement inhibitors.

Anticomplements of Ehrlich and Bordet.

Ehrlich and Bordet by immunizing animals with normal serum succeeded in producing serums highly antagonistic to complement action. According to the Ehrlich conception, these bodies are to

be regarded as distinct antibodies, receptors of the first order. Moreschi, however, has thrown doubt on the existence of such distinct antibodies for the reason that in using normal serum as complement a mixture of protein substances is used giving rise, in immunization, to antialbuminous bodies which react with the antigen to form combinations which inhibit or bind complement. Such combinations in the form of precipitates may be demonstrated to act as anticomplements. The existence of true anticomplements, therefore, while not disproved, has not been satisfactorily demonstrated.

The presence of complementoid in inactivated serum may also act as a cause of complement inhibition by occupying the receptors of the antibody. When these various factors which complicate the complement fixation reaction are considered, it will be seen that great care must be taken in both the technic and the interpretation of results.

Nature of Complement Deviation Antibody.

The substance concerned in antigen, antibody complement fixation is obtained by processes of immunization similar to those concerned in other antibodies. In the one case the reaction is followed by perceptible results, in the other by fixation of complement. The question arises: are the antibodies in these two kinds of reaction identical or not?

That the complement fixation antibody is distinct from precipitins and agglutinins is indicated in different ways. Muir and Martin, by immunizing rabbits with guinea-pig serum, obtained an antibody capable of complement fixation

but which contained no precipitin. Moreschi produced in fowls antiserum which was high in precipitin concentration but did not produce complement fixation. In like manner the presence of antibody complement deviation in the absence of agglutinating properties has been noted. In general, complement deviation antibody is destroyed at higher temperatures than agglutinins.

It is in lytic amboceptors that complement fixation antibody has its closest analogy and opinions are divided as to the identity of the two. Neufeld and Haendel regard amboceptor and complement fixation antibody as distinct from each other and apply the name "Bordet's antibody" to the latter. They reach this conclusion from the fact that if cholera bacilli and their antiserum are mixed with complement and allowed to act at 0° C., hemolytic complement is absorbed but not that necessary for bacteriolysis. The same mixture allowed to act at 37° C. results in an inhibition of action of both complements. The objection to such a proof lies in the fact that the difference may be due to a difference in the complements according to Ehrlich and his school, who believe in the multiplicity of complements. Neufeld and Haendel have also immunized animals with a certain water vibrio and obtained a serum which was bacteriolytic against this vibrio, but not against cholera vibrios. Complement deviation, on the other hand, was effected by using either of the two micro-organisms as antigen.

If Ehrlich's definition of amboceptor as a substance which unites antigen with complement is accepted, it is apparent that the term will apply

to "Bordet's antibody," whether it is identical with other amboceptors or not.

Practical Use of the Reaction.

It will be readily seen that the reaction of complement fixation like other immune reactions between antigen and antibody may be used for the identification of either of the two bodies concerned.

Biologic Test.

The complement fixation test has been used especially by Neisser and Sachs similarly to the precipitin test for the differentiation of proteins.

Animals are immunized to a certain protein and an antiserum is thus obtained. A titration is then made with known homologous antigen and antibody, in varying quantities, to determine the amount of antiserum necessary to produce complement deviation. The quantity of antiserum found by titration to be necessary for complement fixation in the presence of homologous antigen, is then added to the serum or protein to be tested. If this protein and the antiserum are homologous, a complement fixation will result. The method has been criticized because of its extreme delicacy. It is estimated that amounts of protein substances will give a complement fixation test which are present in one-millionth the quantity required for a precipitin test. Uhlenhuth advises the control of the method by the precipitin test. Although other antigens such as bacteria may be identified by complement fixation, the existence of easier methods makes complement fixation of little value.

As Antibody Test.

The existence of easier methods of diagnosis results in infrequent use being made of the complement deviation reaction as a means of diagnosis in infections with organisms capable of cultivation. The presence of the reaction has been

demonstrated, however, in a large number of infections—typhoid, paratyphoid, streptococcus infections (including streptococcus infections of scarlet fever), pneumonia, dysentery, diphtheria, etc.

Kolle and Wassermann have demonstrated complement fixation in meningitis and suggest the use of the reaction to determine the strength of meningococcus antiserum.

Regarding the presence of antituberculin in the blood of tuberculous animals and man, conflicting results are reported. It would appear that complement fixation reaction is inconstant during the course of tuberculosis and that the reaction occurs more constantly after the use of therapeutic inoculations of tuberculin and especially of emulsions of tubercle bacilli.

Complement Deviation in Syphilis.

It occurred to Wassermann that by using extracts of tissues containing antigen, complement deviation antibodies might be found in infections in which the antigen could not be cultivated. By the use of syphilitic tissues he immunized apes against syphilis and by using such syphilitic tissues as antigen found complement deviation antibodies in the serum of the immunized apes.

Wassermann, Neisser and Bruck, in 1906, published the results of these experiments together with a method adapting them to the serodiagnosis of syphilis which has become commonly known as the Wassermann reaction. Since the spirochetes of syphilis were known to be found in extreme numbers in the liver of the syphilitic fetus, an aqueous extract of this organ was used as antigen. To this extract was added the inactivated serum

of the suspected case and after the addition of complement, the mixture was incubated an hour. Sheep's corpuscles with specific amboceptor were then added and the mixture again incubated. In case the syphilitic antibody was present, complement became bound and no hemolysis took place upon second incubation. Through a large number of tests the Wassermann reaction has been proved to be characteristic for syphilis.

Nature of the Antigen of Wassermann.

The idea of Wassermann that the complement binding was due to extract of spirochetes as antigen and specific amboceptor, soon found opposition. Landsteiner, Müller and Poetzl, Levaditi and others showed by the use of alcoholic extracts of normal organs that a substance could be obtained which acted as antigen and could be substituted for the extract of syphilitic liver without changing the results of the reaction. Finally it has been shown that mixtures of lipoid substances or crude tissue lecithin could be used as antigen. Wassermann pointed out that whereas his aqueous extract of syphilitic tissues was thermolabile at boiling, the alcoholic extract of normal organs was thermostabile: further that the aqueous extracts of normal organs do not act as substitutes for aqueous extracts of syphilitic organs. He therefore regarded his original ideas as to the specificity of antigen as correct and held that aqueous extract of syphilitic liver was the only extract which could be used without chance for error.

Seligmann and Pinkus have made a study of the various extracts and conclude that the difference in heating aqueous and alcoholic extracts is

due to the fact that in the aqueous extracts, a large amount of protein is present with the lipoids on which the antigenic action depends, and that these lipoids become bound to the protein through heating; further, they believe that these lipoid substances may be extracted with water in syphilitic liver because through degenerative processes they become split off, while in normal organs they must be split off by alcohol and heat.

They conclude that the antigen is therefore of non-specific lipoid nature and that it acts as activating the complement binding property of syphilitic serum.

The nature of the substance in the serum of syphilitics which in combination with antigen inhibits complement action, is still unknown. Noguchi has shown that it begins to show the effects of heat at 45° C. At 56° C. it is somewhat diminished in activity and at 62° C., its activity is lost. It has been thought of as an antibody because of its development with the development of the disease and its disappearance with the cure by specific treatment.

Technic. As was stated in the beginning of the chapter, the various materials used in complement deviation may of themselves have anticomplementary properties. In addition, it might be stated that extracts of organs may also have a hemolytic action. The importance of quantitative relations and contral experiments will therefore be apparent. The Noguchi modification of Wassermann's method has given satisfactory results in a large number of cases. The preparation of materials

required for the test will be described and then their application to the test.

Sheep's corpuscles may be obtained from the jugular vein of the animal. The blood is defibrinated and washed by centrifugation with salt solution, the salt solution being changed twice. A 5 per cent. suspension of these corpuscles in 0.85 per cent. salt solution is used for the test.

Inactivated hemolytic serum for sheep's corpuscles is prepared by immunizing a rabbit against sheep's corpuscles. The animals should be injected intraperitoneally with washed corpuscles made up to the volume of blood from which they were taken, by the addition of salt solution. At least four or five injections should be given at intervals of from four days to a week, and with amounts beginning with 2 c.c. and ending with from 12 to 20 c.c. The animal should not be bled before ten days after the last injection. Blood is obtained from the marginal vein of the ear and allowed to clot. The serum is then removed, heated to 56° C. for one-half hour and standardized as follows: Varying graded amounts of the inactivated serum plus 0.1 c.c. of complement (fresh guinea-pig serum, best obtained by aspiration from the heart), are added to each of a number of tubes containing 1 c.c. of 5 per cent. sheep's corpuscle suspension. The tubes are incubated for one hour and that tube noted in which the smallest amount of amboceptor (inactivated rabbit's serum), produced complete hemolysis. This is called the hemolytic unit of amboceptor.

The antigen is prepared by the extraction of minced syphilitic or normal liver, with 10 volumes

of 95 per cent. alcohol for a week at 37° C. This extract is filtered and evaporated by means of a fan at a temperature below 40° C. The residue is extracted with ether and the ether evaporated. This residue is again taken up in ether and fractionated twice with acetone to remove the acetone-soluble hemolytic substances. The acetone-insoluble residue is evaporated to dryness and extracted with 95 per cent. alcohol. This solution is used as a stock antigen and suspensions in salt solution are used as material for tests.

A titration of antigen should then be carried out as follows: A serum from a known case of syphilis should be obtained and 0.1 c.c. of the inactivated serum added to each of a series of tubes. Another series of controls is made by the use of a like quantity of inactivated normal serum. To each of the tubes of the test series graded varying amounts of antigen are added and like amounts added to the control tubes. Complement 0.1 c.c. is added to each of all of the tubes and the set incubated one hour. At the end of this time, two units of amboceptor for sheep's corpuscles and 1 c.c. of 5 per cent. suspension of sheep's corpuscles are added and the tubes incubated for another hour. The smallest amount of antigen which, with the syphilitic serum, will bind complement, is indicated by the tube containing the lowest quantity of antigen in which hemolysis has not taken place. The control tube containing a like quantity of antigen but with normal serum should be completely hemolyzed.

Having now standardized both antigen and hemolytic amboceptor, the test of the serum in

question is carried out as follows: 0.1 c.c. of inactivated serum to be tested is added to 0.1 c.c. of complement and that amount of antigen added which was found to be the minimum required to bind complement in the presence of 0.1 c.c. of syphilitic serum. Control tubes are made as follows: One positive control is made as the test serum tube but with a like quantity of inactivated known syphilitic serum in place of the serum to be tested. One negative control is prepared with the same components as the test serum tube except that inactivated normal serum is used instead of the serum to be tested. Three control tubes are made with the test serum in one, positive serum in a second and the normal serum in the third, and salt solution substituted for the antigen to ascertain whether or not the serum alone causes complement deviation. One control tube is made with salt solution instead of serum to see if antigen alone will bind complement. Another control may be made with complement, human serum to be tested and corpuscles to exclude hemolysins for sheep's corpuscles being present in the human serum. After incubation of the antigen, complement, serum mixtures and control tubes for one hour, the hemolytic system, consisting of two units of hemolytic amboceptor and 1 c.c. of 5 per cent. corpuscle suspension, is added to each tube. After a second incubation of one hour the tubes are examined for hemolysis. There should be no hemolysis in the positive control tube. In the other control tubes complete hemolysis should have taken place. If hemolysis is present in the tube containing the test mixture the reaction is nega-

tive, that is, there is no evidence of syphilis. If hemolysis is absent as in the positive control tube the reaction is characteristic of syphilis.

Various modifications of this technic have been used by different experimentors with good results. Noguchi, for instance, has used human blood suspensions with their amboceptor instead of sheep's blood, the purpose being to avoid complications due to hemolysins for sheep's corpuscles found occasionally in human serum. It has been found that a larger number of positive reactions in syphilis can be obtained by using non-heated serum of the suspected patient. It is also found, however, that a larger number of normal inactive serums react as positives; the reaction is therefore not so specific as when the heated serum is used. Synthetical antigens composed of mixtures of lipoid bodies of known composition, have been used with varying degrees of success.

Value of the Wassermann Reaction.

The Wassermann reaction has proved to be of great value in the diagnosis of syphilis. The percentage of positive reactions found in the various stages of syphilis varies with the technic of different observers. The following table is made from a collection of percentages by Pearce:

Stage of Disease	Highest per cent.	Lowest per cent.
Primary syphilis	92.8	64.4
Secondary syphilis	100	71
Tertiary syphilis	100	63
Early latent syphilis	76	51
Late latent syphilis	79	46
Hereditary syphilis	100	86

The percentage of cases in which syphilis could be excluded so far as possible from history and

negative clinical symptoms, and which gave a positive reaction, varies from 0.3 to 3.6 per cent.

The results in the parasyphilitic diseases are as follows: In tabes, using blood serum, the average of positive reactions is 62.88 per cent.; using cerebrospinal fluid, 56.2 per cent. In general paresis, using blood serum, 88.1 per cent. of cases are positive, with cerebrospinal fluid 90 per cent. (Noguchi).

The earliest appearance of the Wassermann reaction is that reported by Lesser, eight days after exposure. The reaction usually appears by the end of the fourth week after the appearance of the chancre.

Among non-syphilitic diseases the Wassermann reaction has been observed in trypanosomiasis, in leprosy with less constancy, in scarlet fever (usually weakly positive and transient), in frambesia, tuberculosis and carcinoma. In these non-syphilitic diseases, the ones giving the highest per cent. positive reactions are those least likely to be confused with syphilis. The reactions found at times in carcinoma and tuberculosis may be due to concurrent syphilis.

Specific treatment has been found by all observers to have a profound influence on the Wassermann reaction and disappearance of the test has been observed after treatment for from six months to five years.

A positive reaction is therefore regarded by many observers as an indication for further treatment.

CHAPTER XVIII.

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-

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 of 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 from twenty-four to forty-eight 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 ani-

Antinephrotoxins.

mal; as a result the receptors were overproduced 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.

Neurotoxins.

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.

With the idea of preparing an antigen which would contain, so far as possible, only substances characteristic of variety of cells used, Beebe has made use of nucleuproteids of various organs, especially thyroid gland, to produce antiserums. His results would indicate that in this way serums may be obtained, which are specific for different varieties of cells except in high concentration.

With the use of such a thyrotoxic serum he has reported good results in cases of hyperthyroidism.

Pearce has failed to confirm these results and obtains results, with neucleoproteid as antigen, which are similar to those which he obtained with simple extracts.

Beebe, however, criticises the technic of Pearce in that Pearce heated his preparation, thus, according to Beebe, destroying the specific biologic character of the extract.

It would appear that further observations are desirable before conclusions can be drawn regarding the value of nucleoproteid as antigen.

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 injected, 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 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.

"Horror Autotoxicus."

CHAPTER XIX.

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

Cytases.

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.

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 Hair.

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 phenomenon which bears the name of phagolysis, i. e., solution, partial or complete, of 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.

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.

Relation of Phagocytosis to Virulence of Bacteria.

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

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 to be definitely established that contaminating organisms (pyogenic cocci, *Bacillus prodigiosus*) may greatly increase the virulence of the bacillus of symptomatic anthrax, *Bacillus Welchii*, and the tetanus bacillus—anaerobic or-

ganisms. 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 appear-

ance. Examination shows an enormous degree of phagocytosis. When, on the other hand, non-immune 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.

The Influence of Serum.

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 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. 324) 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 leu-

cocytes 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

Microcytase is Intracellular.

diffusion. Similar conditions prevail in edematous fluids. In another instance a portion of a vein, 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.

CHAPTER XX.

OPSONINS.

Although the importance of the influence of the serum in phagocytic processes was recognized by Denys and Leclef, it remained for Wright and Douglas to demonstrate that substances exist in the serum which are capable of rendering bacteria susceptible to phagocytosis. The name opsonin which they applied to this substance has come into general use.

The proof of the action of opsonin on bacteria was based on the following facts: 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.

Opsonic Index.

Later experiments showed that the opsonic power of the blood varied in the course of disease just as is found in the case of other immune substances. The relation of such an abnormal opsonic power to that of normal serum was designated as the opsonic index.

Technic.

The Wright technic deals with three factors as will be apparent from the above: leucocytes, bacteria and serum.

Leucocytes.—The leucocytes are obtained in different ways according to the kind employed. Human leucocytes are obtained by puncturing the lobe of the ear or tip of the finger with a small lancet and catching the blood in a 1 to 1.5 per cent. solution of sodium citrate in 0.85 per cent. sodium chlorid solution. The amount of blood necessary is usually small, about 1 c.c. and 10 c.c. of citrate solution is required to keep the blood from clotting. By centrifugalizing, the corpuscles are separated from the citrate solution and the corpuscles washed by pipetting of the supernatant fluid and replacing it with physiologic salt solution. Two such washings are made and then the pearly-colored blood cream containing the leucocytes is removed from the surface of the red cells for use.

Serum.—This is obtained as for agglutination or other tests.

Bacteria.—The bacteria are obtained by growing on the surface of an agar slant for from 12 to 24 hours; they are then removed into salt solution either by means of a loop or by adding the salt solution directly to the agar slant. The concentration should be such that a smear on a slide shows plenty of bacteria to the field of the microscope while at the same time the individual organisms are well separated from one another. Frequently to obtain such a mixture it is necessary to shake the emulsion thoroughly to insure division of clumps.

Having the above constituents they are mixed together in the following way:

A capillary tube is made by drawing out a glass tube of about 4 mm. caliber and a length of about 16 cm. A small volume of serum is allowed to run into the tube by capillary attraction and the length of the volume marked on the outside of the tube. A small air bubble about 1 mm. in length is then drawn into the tube and then a volume of bacterial suspension equal to the volume of serum. Again a small bubble of air is drawn into the tube and lastly a volume of leucocyte mixture equal to those of serum and bacteria.

The three constituents are then mixed together by drawing the three up into the large part of the tube and mixing together there by drawing back and forth or they may be mixed on a glass slide and then drawn back into the capillary tube. The mixture is then incubated the desired length of time (usually about 15 minutes) and smeared on a slide as in making an ordinary blood smear.

The slide is then stained with an appropriate stain (for most bacteria one of the eosinates of methylene blue) and examined with the immersion lens of a microscope.

The number of bacteria in successive leucocytes is counted and an average made. Various figures are given as the necessary number of leucocytes which should be counted to give accurate results. The number, however, should be governed by the uniformity of the numbers of bacteria in successive leucocytes. If, for instance, the average number of bacteria in three successive counts of ten leucocytes is nearly the same, it is more accurate to take such an average than if more leucocytes are counted with no uniformity of numbers.

The ratio of the average number of bacteria to the leucocyte taken up in the presence of a given serum to the average number taken up in the presence of a serum taken as normal is the opsonic index.

Immune Opsonins.

By the immunization of animals by various bacteria and other cells a serum of high opsonic power may be produced. The opsonic action of such serums, in contrast to that of normal serum, is not destroyed by heating to 56° C. and differs from normal opsonin in other respects which will be discussed later. In immunizing animals with typhoid bacilli, it was noticed that the estimation of the concentration of opsonin in the typhoid immune serum by the Wright method of comparison did not show results that would be expected. That is, a highly immune animal would show little difference from one with low immunity. Klien, therefore, estimated the opsonic power by determ-

ining the dilution point at which the number of bacteria taken up by the leucocytes equals the number taken up without the presence of serum. This dilution point is sometimes called the point of opsonic extinction. The phagocytosis taking place without the influence of serum is known as spontaneous phagocytosis.

Specificity of Opsonins.

There has been considerable conflict of opinion as to whether there are specific opsonins in normal serum for different varieties of cells or one opsonic substance capable of acting on a variety of cells. Hektoen concludes from his own studies and those of others, consisting of specific absorption experiments and observations on the specific fall in opsonic power following injection of specific antigen, and from other experiments, that normal serum contains specific opsonins which are capable of specific absorption and which are the same substances which are increased to form the immune opsonin.

The immune opsonins are easily demonstrated by absorption experiments to be highly specific.

Hektoen and Ruediger have shown that normal opsonins are almost completely destroyed or inactivated by heat and are therefore thermolabile. The inactive opsonin (opsonoid) by saturating the receptors of bacteria with the haptophore group prevents further sensitization with fresh serum.

These investigators also show that opsonin may be bound or neutralized similarly to complement by solutions of various salts.

The nature of immune opsonins has been the subject of much discussion. As was stated before, immune opsonins resist a temperature of from 56

to 60° C. Dean, Cowie and Chapin, and others have shown, however, that the opsonic power of heated serum may be increased by the addition of normal serum similar to that reactivation taking place on adding complement to amboceptor. Browning has pointed out that this apparent similarity of the action of normal serum on heated opsonin may be due to summation of effects. He has shown that by separating immune body in opsonic serum at 0° C. by saturation with bacteria and then adding complement there is a true activation, and that no such action occurred in treating the bacteria with complement, washing and then adding heated opsonic serum. He concludes that immune body and complement may be concerned in opsonic action, but leaves open the question of whether the immune body is the thermostabile opsonin or not.

Opsonins as Distinct Antibodies.

Hektoen concludes from the following facts that opsonins are distinct from other antibodies.

1. Heat may almost completely destroy the opsonic power of serum leaving the lytic amboceptors intact.

2. Serum, normal as well as immune, may contain opsonin for a given organism but not, at least so far as is known, the proper lytic amboceptor for that organism.

3. A serum may contain opsonin for an organism, but no agglutinin and the opsonin may persist after destruction of bacteriolytic complement by heat.

4. In immunization lytic and opsonic powers do not run parallel.

Relation to Immunity Processes.

If an animal is injected with a proper dose of bacteria or alien red cells, there results as a rule in the first day or so a fall in the opsonic content of the blood along with other antibodies. This period is known as the "negative phase," and is followed by a steady rise which reaches its height from the eighth to the twelfth day and gradually falls to normal. The negative phase as pointed out by Hektoen is specific and it has not been determined whether it is due to a specific absorption or to an effect on the antibody producing cells.

"In several acute infectious diseases the course of the formation of new opsonin for the infecting agent, in the typical attack, terminating promptly in recovery without complications, shows a marked general resemblance to the opsonin and antibody curve after a single antigen injection in the normal animal; it also bears definite and constant relations to the clinical phenomena. During the early stages when the symptoms are pronounced there is a negative phase and then as the symptoms begin to subside the opsonin curve rises above normal, reaching the highest point several days after the onset, followed by a gradual subsidence. This is true of the pneumococcus opsonin in pneumonia, of the opsonin for the diphtheria bacillus in diphtheria, of the streptococcus opsonin in erysipelas, and also of the opsonin for the diplococcus of mumps in that disease. The curve is typical also for the streptococcus in scarlet fever, indicating clearly that this organism unquestionably plays a definite rôle in scarlet fever, whatever its actual causative relation to the disease may be.

In pneumonia the greatest rise in the leucocytosis appears to precede somewhat the highest rise of the opsonin. In all these diseases the typical wave-like opsonin curve is modified by the development of complications of various kinds and at the onset of which it commonly undergoes a distinct depression. In rapidly fatal cases, for instance of pneumonia, the opsonic curve or index may not return from the primary depression, but sink lower and lower. In prolonged infections, general as well as local, there occur irregular fluctuations and in chronic, more or less stationary cases, the opsonic index is often subnormal. At this time further details cannot be given. My chief point is to make clear the close association between recovery and the wave-like rise of the opsonin, and, as a result of the immunization in all likelihood also of other antibodies, in the typical attack of acute so-called self-limited infections. In some of the diseases the opsonin is the only antibody that we can measure readily with our present means. As I have stated, an intraphagocytic destruction of pneumococci and streptococci takes place in the presence of fresh leucocytes and opsonic serum, whereas either alone constitutes a good medium for these bacteria. Taking these facts into account it seems to me that the wave-like course of the opsonin in pneumonia and in acute streptococcus infections is a strong point on the side of the signal importance of phagocytosis in their healing, whatever other measure, of which at present we know less or nothing, may be in operation also."[1]

1. Hektoen: Opsonins and Other Antibodies, Science, 1909.

Interaction of Action of Leucocytes and Opsonins.

As pointed out by Glynn and Cox, the work of Wright and his followers has resulted in an undue neglect of the importance of the variation in the power of leucocytosis in the leucocytes themselves as a factor in phagocytosis. They emphasize the fact that while the determination of opsonic power may be an indication of the degree of immunity, it does not represent the phagocytic power of the blood as a whole. In order to ascertain the valuation of the different components separately and as a whole they suggest the comparison of the leucocytes of the blood in question with those of normal blood and call the ratio of the first to the second the cytophagic index. Secondly, they compare the action of the leucocytes and the serum of the blood in question with the action of normal leucocytes and serum. The ratio of the first to the second is called the opsonocytophagic index.

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 pro-

duce 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 leukocytes, 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.

AGGRESSINS

Aggressins. 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 leukocytes 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 unknown, 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.

Proof of the non-identity of the aggressins of Bail and the toxins produced by the organism has not been very convincing.

Virulin. Investigating the resistance of virulent pneumococci (which vary greatly from non-virulent forms) to phagocytosis, Rosenow was able, by autolysis in salt solution, to extract the substance on which this resistance depends. He was not only in this way able to render them phagocytable, but also by treating non-virulent strains with this extract he was able to render them more virulent and resistant to phagocytosis.

The substance which he calls virulin is insoluble in alcohol and ether, and is thermostable.

CHAPTER XXI.

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.

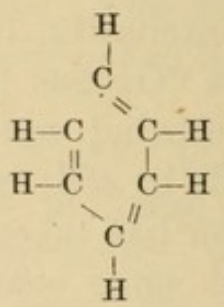
"Leistungskern" and Side-Chains.

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."*

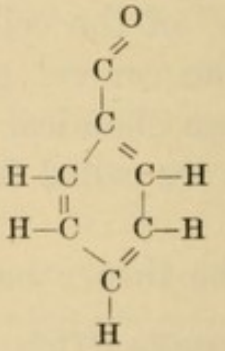
The author of the theory has made his concep-

1. Ueber das Sauerstoffbedürfnis des Organismus.

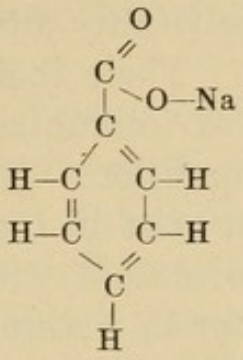
tion more tangible through an analogy which was drawn with the so-called ring or nucleus of benzol and its side-chains. The molecule of benzol, C_6H_6, has a definite formation in which each carbon atom is linked to two others in such a manner as to form a ring; three valences of each carbon atom are 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 XII experiments were cited to show that antitoxin does not destroy the toxin, and this is generally admitted to-day. 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 XII, 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 Amboceptor 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 spider-crab) 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 XI.

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 newformed 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-

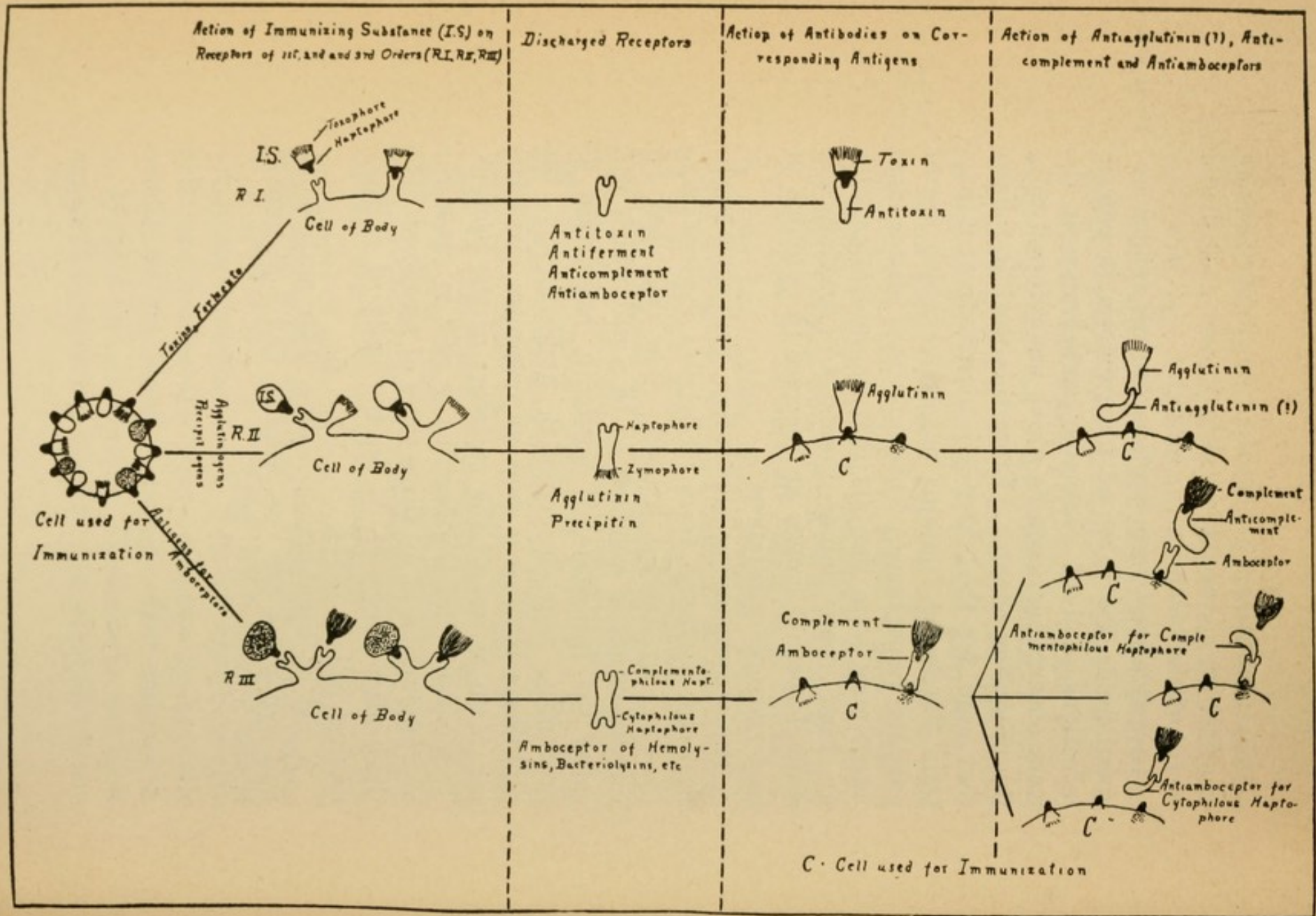
cytosis would seem to be of prime importance in the first group, and the antitoxic and bactericidal power of the serum in the second.

Opsonins.

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.

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. 360).

A list of immunizing bodies, their anti-bodies, and synonyms for complement and amboceptor, is also appended (p. 361).


Action of Immunizing Substance (I.S) on Receptors of 1st, 2nd and 3rd Orders (R.I, R.II, R.III)
Discharged Receptors
Action of Antibodies on Corresponding Antigens
Action of Antiagglutinin (?), Anticomplement and Antiamboceptors
I.S.
Toxophore
Haptophore
R I.
Cell of Body
Toxin
Antitoxin
Antitoxin
Antiferment
Anticomplement
Antiamboceptor
Toxins, Ferments
Cell used for Immunization
Agglutinogens
Precipitogens
R.II
Cell of Body
Haptophore
Zymophore
Agglutinin
Precipitin
Agglutinin
C
Agglutinin
Antiagglutinin (?)
C
Complement
Anticomplement
Amboceptor
C
Antigens for Amboceptors
R III
Cell of Body
Complementophilous Hapt.
Cytophilous Haptophore
Amboceptor of Hemolysins, Bacteriolysins, etc
Complement
Amboceptor
C
Antiamboceptor for Complementophilous Haptophore
C
Antiamboceptor for Cytophilous Haptophore
C
C · Cell used for Immunization

LIST OF IMMUNIZING BODIES AND THEIR ANTIBODIES.

Antigens or Immunizing substances.	Products of immunization.		
Toxins.	Antitoxins.		
Complements	Anticomplements		
Ferments.	Antiferments		
Precipitogenous substances	Precipitins		
Agglutinogenous substances	Agglutinins		
Opsonigenous 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 XXII.

PRINCIPLES OF SEROTHERAPY.

In the strict sense serotherapy means the injection of antitoxic or antibacterial serums for curative or prophylactic purposes; this is passive immunization or direct serotherapy. 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 serotherapy. 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.

General Strength of Serums.

In general, serums to be effective must have a 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 cubic centimeter.

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

1. See Chapter XI (Part II).

other. There is no third substance with which one or the other may unite. In the body, however, the 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.

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.

Nature of Curative Action.

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 recupera-

tive 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 ANTIBACTERIAL 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 represent-

atives, cause the development of strong bactericidal serums in the immunized animal. In Chapter XVI, 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 simultaneously 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, thereby liberating an excessive amount of endotoxin, 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 stimulating phagocytosis. It has been shown 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 streptococcus 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-organisms 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.

Mixed Active and Passive Immunization.

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.

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.

Curative Vaccination.

Wright, following his observations on the variations in opsonic power of the serum in different infections, concluded that in certain localized chronic infections such as chronic suppurative processes, the body as a whole did not respond to the infection with the production of antibodies. The injection of dead homologous organisms was therefore resorted to in order, in the words of Wright: "To exploit in the interests of the infected tissues, the unexercized immunizing capacities of the uninfected tissues."

The dosage of the injected vaccine was determined according to the purpose of using the minimum quantity which would result in the maximum response of the uninfected tissues with the least development of the so-called negative phase. In order to regulate the frequency and size of the therapeutic inoculations, Wright made use of opsonic index estimations.

Vaccines are prepared as follows:

The desired organism is grown on a suitable solid medium as an agar slant or blood serum slant for the minimum time required for a good growth, usually twenty-four hours.

Salt solution is then added to the slant, a few cubic centimeters are usually sufficient for an ordinary growth (the quantity need not be exact), and the culture scraped off into the salt solution with a sterile glass rod or platinum loop. The number of bacteria per cubic centimeter is then estimated by mixing equal volumes of defibrinated

blood and bacterial suspension and then diluting and smearing on a slide. The dilution should be about five times. The method of measuring equal small volumes by means of capillary tubes as given in Chapter XIX, may be used and a Romanowski stain used for staining.

Since the number of corpuscles in normal human blood is about 5,000,000, by a comparison of the number of bacteria with the number of corpuscles the number of bacteria per cubic centimeter can be readily estimated. By dilution with salt solution the required dosage per cubic centimeter may be obtained. When it is necessary to keep the vaccine a small amount of tri-cresol (.2 per cent.) may be added.

Recently therapeutic inoculations have been used for a great variety of infections, both acute and chronic, and to both local and systemic infections. Naturally, the results have also varied within wide range.

In case of chronic localized infections the theoretical basis for the use of vaccines seems plain. As pointed out by Theobald Smith: "The effectiveness of vaccines applied in the course of acute febrile diseases, such as typhoid fever and pneumonia must be accounted for by principles of which experimental medicine has as yet no definite knowledge." Theoretically no advantage can be expected from adding toxins in an already over-intoxicated case.

This criticism does not apply, of course, to such a procedure as is suggested by Rosenow. (See chapter on Pneumonia.)

CHAPTER XXIII.

ANAPHYLAXIS.

Attention has already been called to the fact that an individual may be more susceptible to infections at one time than at another through various accidental conditions, as exposure and exhaustion. This, however, is not a specific hypersusceptibility and is usually more or less transient.

In contrast to this accidental condition, stands a specific susceptibility which is now commonly known as anaphylaxis. The condition of the individual or animal is spoken of by V. Pirquet as allergy (*Allergie*), a word which conveys the idea of an altered power of reaction on the part of the animal body.

V. Pirquet's conception of allergy is best described in his own words, in which he uses vaccination and revaccination as an illustration. "Vaccinia, with which we can at any time institute an infection, is just as much an infectious disease as is variola, of which it represents an attenuated form. Let us inoculate one person who was vaccinated two years previously and who, according to the customary view, is immune, with a drop of lymph. Then inoculate another who has not gone through this process and attend it closely. Now will the immune person show absolutely nothing? On the contrary, when we return after 24 hours, we find in the one who received his first inoculation (the normal person), a small crust showing no reaction, while in the

immune there is a normal small, raised, inflammatory, itching, hyperemic area.

"Is the one previously inoculated, therefore, hypersusceptible? If we wait a few days the picture changes: The papule becomes brownish and smaller, while in the case of the first vaccination, a vesicle forms under the crust, which increases more and more, becomes surrounded by a wide zone, and leads to a pustule. Now we must conclude that the one receiving his first inoculation is the more susceptible, since he has fever, pain, and a marked local inflammation, while in the immune person signs of infection have long since disappeared.

"As it appears to me both individuals have reacted: The one earlier, the other later; one with a papule, the other with a pustule. In one the reaction was hardly noticeable, in the other pronounced. Through the previous inoculation no immunity in the sense of insusceptibility has developed, but it is only the ability to react which has changed, and this in point of time, quality, and quantity."

The condition then appears to be a paradoxical one, in that we have a certain degree of hypersusceptibility in a person who is really immune and his immunity may depend to a greater or less degree on his ability to react quickly to the presence of the infectious agent before the latter has time to proliferate extensively.

Portier and Richet, in 1902, observed a peculiar behavior on the part of a poison found in certain actinia.

The poison was invariably fatal for dogs when given as a first injection in doses of 0.8 gm. per kilogram of animal weight, but was rarely fatal in doses of under 0.2 gm. per kilogram. Death usually occurred in from four to nine days. When a second dose, however, was given to an animal which had recovered from the first injection, death supervened in a short time, usually within two and three-quarters hours. The first injection evidently modified the resistance of the animal in some way so that it became more susceptible to the poison than it was in the first instance. It was to this modified state of the animal's resistance that Richet applied the name "anaphylaxis," which stands in contrast to a condition of prophylaxis.

Arthus' Phenomenon.

In 1903, Arthus observed that, when rabbits had received several injections of horse serum at intervals of several days, the serum ceased to be absorbed as at first and that there resulted local necrosis and often sloughing with subsequent ulcer formation.

Theobald Smith's Phenomenon.

In 1904, Theobald Smith told Ehrlich of a phenomenon which he had observed while testing the potency of diphtheria antitoxin on guinea-pigs. Animals which had received injections of antitoxic horse serum and later were injected with a small quantity of normal horse serum became acutely ill or died.

In the following year, 1905, appeared the articles of Otto, Rosenau and Anderson, and of v. Pirquet and Schick. Since these articles an enormous amount of work has been done to cor-

relate the phenomena of anaphylaxis with other processes of immunity.

Like other processes of immunity anaphylaxis may be classified as natural and acquired; and, again, acquired anaphylaxis may be active, in which the process results from a reaction on the part of the tissue cells, or may be passive—resulting from the introduction of ready-made substances into the body.

Natural Anaphylaxis.

It has been long known that, as noted by Horwitz, Schofield, Doerr, and others, certain individuals are unusually affected by the ingestion of eggs, crabs, flesh, pork, etc. The symptoms are variable, but there is often nausea, fever, colic, and exanthemata. It is of course to be questioned as to whether such hypersusceptibility is really natural or acquired by early sensitization. Schofield reports such a case of hypersusceptibility to egg which disappeared after repeated increasing doses of egg taken in pills, using minute amounts to begin with. Such hypersusceptibility has been long known under the name idiosyncracy. The idiosyncracies to proteins, however, should be distinguished from those in which known chemical substances, such as mercury or salicylic acid, are concerned.

Active anaphylaxis has been studied in a variety of mammals and fowls, and substances which correspond to those concerned in immunization have been demonstrated.

It will be well to take up these various factors, and after a discussion of their nature, it will be easier to understand the theories concerning the mechanism of their action.

Antigen.

The sensitizing agent in anaphylaxis has received the name of anaphylactogen, or sensibilisinogen, and may be defined as any substance which, when taken into the body, produces a specific hypersusceptibility, usually after an incubation period of at least from five to seven days.

The substances which have been demonstrated to act as anaphylactogens are proteins or are inseparably connected with proteins. That anaphylactogens are closely related, or, as Friedberger, Doerr, and others think, identical with those bodies which produce complement deviation, antibodies, and precipitins, is shown by the fact that the same substances produce all three phenomena. Thus, as with precipitinogens we have an anaphylaxis specific for species and for tissues. The same tissues which are specific for precipitin formation, crytalline lens, spermatozoa, and placenta, also give a specific anaphylaxis, while those tissues such as kidney, liver, etc., which produce only species specific precipitins produce species specific anaphylaxis. As an exception to this rule, it may be mentioned that Wells does not find that iodized albumin produces anaphylaxis specific for iodized albumins rather than species specific reactions, as was found for precipitins by Obermayer and Pick.

Relation to Primary Toxicity.

One of the first questions which arose concerning Theobald Smith's phenomenon was that of the relation of the anaphylactogen to the diphtheria toxin and antitoxin. In this case, the employment of normal horse serum readily showed that anaphylaxis was independent of the diphtheria bacillus derivatives and antitoxin present in the serum.

Somewhat more difficult is the question regarding the primary toxicity of such substances as are contained in eel serum, various phytalbumins and bacteria. It has been shown by Doerr and Raubitschek that by heating or by acidifying eel serum it is possible to remove the primary toxicity without taking away the property of producing anaphylaxis. In a similar way it has been shown by Rosenau and Anderson, Vaughan, and others, that bacterial proteins free from toxic action can produce anaphylaxis. It has also been shown that whereas in primarily toxic serum the larger the dose the greater the toxicity, in anaphylaxis sensitization smaller doses sensitize more readily than large ones.

That hypersusceptibility to true toxins does occur, however, has been demonstrated in the case of diphtheria toxin and tetanus toxin. The phenomena here, however, are distinct from anaphylaxis in the fact that the incubation period is absent, that the symptoms come on gradually after the second dose, and lastly, that after a certain length of time which corresponds to the incubation time of anaphylaxis, immunity or decrease in susceptibility, occurs in contrast to anaphylaxis. We must conclude, then, that toxicity and sensitizing properties are distinct from each other.

Sensitization and Toxicity.

The anaphylactogen comes into consideration in the primary or sensitizing dose and in the secondary or toxic dose. Many experiments have been carried out to determine whether or not the sensitizing and toxic action are dependent on the same substance or similar qualities of the same substance. It was found, for instance, that in the

case of egg albumin one twenty millionth of a gram sufficed for sensitization (Wells), and about one thousand times that amount was required for a toxic dose. It was also found that by heating to 90° or 100° C. it became much more difficult to cause intoxication in sensitized animals than to sensitize them. Besredka concluded from these experiments that sensitizing and toxic substances were distinct from each other. Vaughan and Wheeler by digestion with hot absolute alcohol and sodium hydrate obtained a separation of albumin into two parts, one of which showed a marked sensitizing property and but little toxicity (alcohol insoluble portion); the other portion (alcohol soluble), a more highly toxic action. They concluded that toxic and sensitizing substances were present in the same molecule and that by their process a splitting of the molecule into toxic and sensitizing groups was obtained.

The work of Vaughan and Wheeler was done on whole egg white and other crude proteins.

Wells, working with pure crystallized albumin, obtained toxic and sensitizing action in this way in quantities smaller than those represented by Vaughan's minimum sensitizing and toxic split products. It seems possible, therefore, that by the process of Vaughan and Wheeler amounts of protein too small to produce toxic effects, but capable of sensitizing, escaped splitting through alcohol precipitation while the alcohol soluble portion consisted of toxic split products. The action of heat may be due to the fact that both toxic and sensitizing substances are equally influenced, but that the apparent effect is greater on toxicity because

for intoxication larger amounts are necessary than for sensitization.

Wells has also shown that toxicity and sensitizing properties decrease equally by tryptic digestion, and that both disappear with the disappearance of heat coagulable proteins. According to Wells, the importance of the action of heat is due to the coagulation of protein, thus rendering it capable of being taken up and digested by the leucocytes. Casein and other proteins which do not coagulate on boiling suffer no change through heat until a temperature which destroys the protein molecule is reached.

That toxic and sensitizing substances may be closely related to the aromatic groups of the protein molecule is suggested by the fact that gelatin which is devoid of tyrosin and contains little of other aromatic groups does not produce the phenomena of anaphylaxis (Wells).

Anaphylactic Antibody.

By the injection of serum of sensitized animals it is possible to produce a passive sensitization, analogous to passive immunity, and in this way to demonstrate the presence of an anaphylactic antibody. This antibody has received the name of "anaphylactin" (Rosenau and Anderson), or "allergin" (Anderson and Frost). It is possible to produce passive anaphylaxis in various animals, but, as in active anaphylaxis, the guinea-pig is best adapted to the purpose. Transmission of anaphylaxis from one species to another is also possible. From rabbit to guinea-pig anaphylaxis is readily transmitted.

The necessary interval of time elapsing between the injection of the serum containing anaphylactic

antibody and the actual sensitization of the animal varies with the different methods of injection. In case of intraperitoneal injection this time is about twenty-four hours; in intravenous injections it is about one and one-half hours.

Different means of measuring the sensitizing strength of antiserums have been suggested. Doerr and Russ injected decreasing quantities of the serum into guinea-pigs and then by injecting twenty-four hours later an intoxicating quantity of antigen into each of these pigs, the amount of antiserum necessary to produce sensitization was found. A second method is to inject a definite quantity of antiserum into each of a series of pigs and then twenty-four hours later to inject decreasing quantities of antigen to find the smallest amount necessary to cause acute death. Doerr and Russ suggest as a unit of anaphylaxis antiserum or of anaphylactin such a serum as will in a dose of 1 c.c. intraperitoneally sensitize a 250 gm. guinea-pig so that acute death may be produced in twenty-four hours by injecting a sufficient quantity of antigen.

Relation to Other Antibodies.

The close connection between anaphylactogen and precipitinogen has already been alluded to. In a similar way, anaphylactin and precipitin are so closely allied that Friedberger, Doerr, and others consider them identical. As objections to this view it is pointed out that animals which do not readily produce precipitins, such as guinea-pigs and dogs, are most susceptible to sensitization; and secondly, that in the state of antianaphylaxis, to be described later, precipitins may be present, but apparently the anaphylactin is exhausted.

The anaphylactic antibody is thermostabile as are precipitins and agglutinins. That is, it resists a temperature of 56° C. for one-half hour.

The Role of Complement in Anaphylaxis.

Michaelis and Fleischmann observed that during and after anaphylactic shock the serum of the animal became poor in complement. Sleeswijk found that following the second antigen injection, complement began to disappear after five minutes, and this disappearance became marked in thirty minutes. In cases in which death took place quickly and complement disappearance was not yet far advanced a further disappearance could be found by allowing the serum to stand for a while in the test tube.

That the disappearance of complement is in itself not responsible for the anaphylactic shock is shown in two ways: first, the injection of complement before or after the second antigen injection does not prevent shock, and secondly in most rapidly fatal shock, death takes place before complement has disappeared to any extent. Friedberger and Hartoch have also shown that injection of complement-binding salt solution inhibits anaphylactic symptoms, when it is injected before the second injection of antigen (see Chapter on Complement Deviation).

Anaphylotoxin.

In order to study further the relation of complement to anaphylaxis, Friedemann sensitized rabbits to ox-blood corpuscles and then added inactivated serum from these rabbits to ox erythrocytes in the test-tube. He found that when complement was added to such a mixture, it became toxic and capable of producing anaphylactic symptoms. By preventing complement binding, by

complementoid, etc., the formation of toxin was prevented. Friedberger was able in a similar way to produce substances which caused symptoms of anaphylaxis, by treating precipitinogens from various sources with precipitins in the presence of complement. He therefore supposes that this toxic substance, which he calls anaphylatoxin, is derived from the precipitate caused by precipitin acting on precipitinogen, and that it is the specific cause of intoxication in anaphylaxis. As there exists a difference of opinion as to the identity of anaphylactin, so there exist various theories as to the formation of anaphylatoxin.

Theoretical Considerations.

Richet supposed that anaphylactic antibody and antigen combined to form the poisonous substance which he called "apotoxin." Wolff-Eisner, Weichhardt, Friedemann and others consider anaphylactin to be of the nature of a lytic amboceptor, and that, by the action of complement through this amboceptor, a splitting of anaphylactogen into toxic substances takes place. Vaughan and Wheeler, with others, consider that in sensitization we have to do with the development of specific proteolytic ferments which split the antigen into toxic groups similar in nature to their toxic products obtained by hydrolysis with alcohol and sodium hydrate. This view is supported by the fact that Biedl and Kraus have produced symptoms of anaphylaxis by injection of split products of protein (Witte's peptone) in dogs. The production of increased protein splitting power of the serum after injection of foreign proteins as demonstrated by Abderhalden also supports the enzyme theory. According to these views, various

anaphylatoxins are of similar character but formed through the action of substances which are of specific nature.

Symptoms of Anaphylaxis.

The symptoms of anaphylaxis vary with different animals. It is in the guinea-pig that most constant results are obtained. Symptoms begin at different intervals of time, after the second injection, with different proteins. With animal proteins, they appear in about fifteen minutes after intraperitoneal injection. The symptoms usually appear somewhat later with vegetable proteins. The animal becomes restless, there is a tendency to scratch, the hair stands on end, and difficulty in breathing comes on. Paralysis of the hind legs is common with animal proteins but is less common in plant proteins. The respiration becomes spasmodic, the animal is unable to stand, convulsive movements occur, and death follows rapidly when a fatal dose is given. When a non-lethal dose is given symptoms may be delayed for an hour. Death commonly occurs in fatal cases inside of an hour and often in less than half an hour. In intravenous and intracardiac injections, the symptoms follow much more rapidly than in intraperitoneal injections. In subcutaneous injections, the symptoms occur long after injection and are inconstant and much less severe than with other ways of absorption. Fatal results are much more difficult to produce in subcutaneous injections.

The blood pressure is raised and lowering does not take place until shortly before death. A very important symptom is that described by Pfeiffer, who observed a constant sudden drop in tempera-

ture. This symptom is considered of great diagnostic value when the drop amounts to 2° or 3° C. and other experimental conditions are constant.

In dogs, the symptoms vary greatly from those in guinea-pigs. Here a characteristic fall in blood pressure is found. Vomiting, involuntary urination and defecation, paralysis and narcosis are common symptoms.

The respiratory mechanism of shock in guinea-pigs, according to Auer and Lewis, is that of a spasmodic contraction of the unstriped muscle of the bronchioles resulting in obstruction of the lumen, acute emphysema and death from asphyxiation.

Section of the vagi does not influence this pulmonary phenomena, indicating that the effect is a peripheral one. Schultz has shown that the unstriped muscle fiber of a sensitized guinea-pig contracts more vigorously when specific serum is applied directly to it than when other serum is used. Atropin, which acts on the nerve endings, causes an inhibition of symptoms. Marked congestion of the abdominal blood vessels is a common finding at autopsy. Various hypnotic and narcotic drugs have been described as inhibiting anaphylactic symptoms, but the effect seems mainly due to a masking of symptoms.

Antianaphylaxis.

A sensitized animal which has recovered from a non-lethal toxic dose of protein is refractory to the action of a later dose. The condition of such an animal is known as antianaphylaxis, and it has been demonstrated by failure to produce passive anaphylaxis by using the serum of an animal in a state of antianaphylaxis, that the condition

is due to an exhaustion of anaphylactic antibody. In animals which have been actively sensitized, the period is a transient one, followed by a return of hypersusceptibility due to continued production of antibodies. In passively sensitized animals, as there is no further source of antibodies, the result depends on the amount of antigen injected. If, for instance, the animal is insufficiently sensitive to allow of death from a large toxic dose, the antigen remaining will produce an active sensitization. If the antigen is just enough to neutralize the anaphylactin, the animal will then be, as before, sensitized. If the second dose is too small to neutralize the anaphylactin, that which remains will be capable of producing further reactions. In animals immunized to proteins and which have a high concentration of antibodies in the blood, so that passive anaphylaxis may be transmitted to a second animal by the use of a small quantity of serum of the immune animal, a state of antianaphylaxis may be produced by injections of amounts of antigen too small to produce a fatal result. In this case, we have an animal which is antianaphylactic although possessing a serum containing a high concentration of anaphylactin. Friedberger supposes that in this case the receptors of the cells are occupied by anaphylactic antibody which is not reached by the injected antigen, this being neutralized by the circulating anaphylactin.

Tuberculin Hypersusceptibility.

The relation of the tuberculin reaction to anaphylaxis (see Tuberculosis) has been the subject of much discussion.

Yamanouchi succeeded in producing anaphylactic symptoms in guinea-pigs by sensitizing them

with serum from tuberculous patients and then, twenty-four hours later, injecting tuberculin or tubercle bacillus emulsion. Bail passively sensitized guinea-pigs by the injection of tuberculous tissues and in this way obtained anaphylactic symptoms by injecting tuberculin after twenty-four hours. Helmholtz produced passive sensitization against cutaneous reaction by the injection of serum from tuberculous guinea-pigs into normal guinea-pigs so that after twenty-four hours, they gave a positive v. Pirquet test.

In contrast to the results of Yamanouchi and Bail, other investigators have succeeded in passive sensitization of guinea-pigs by injection of serum from tuberculous animals either only occasionally or not at all. Production of anaphylaxis by active sensitization with tuberculin is possible only after repeated large doses. It would seem, therefore, that as in the case of other bacteria, typhoid, dysentery, etc., the anaphylaxis is against the proteins of the tubercle bacillus rather than the toxin produced by it.

Serum Disease.

The untoward symptoms following the injection of curative serums has been the subject of study by many investigators, particularly v. Pirquet and Schick, Rosenau and Anderson, and Weaver.

The reaction following a primary injection of serum appears after a period of time varying from a few minutes to several weeks. There may be slight redness and itching at the inoculation site, and swelling of the adjacent lymph glands. The prominent symptoms are fever, skin eruptions, edema and joint pains. Slight albuminuria and leukopenia have been noted.

The reaction varies in severity with the amount of serum used but individual variation and differences in the serum are the most important factors. The reaction following a second injection v. Pirquet and Schick divide into (a) immediate, appearing after a few hours, or (b) delayed, appearing after a few days or a week. The symptoms are similar to those following a primary injection but are more likely to be severe and may be accompanied by vomiting, convulsions, collapse and, rarely, death.

The abnormal reactions following a second injection are more likely to appear when the patient has had a reaction after the primary injection; secondly, when large amounts of serum have been given in the primary injection; and thirdly, with a history of asthma (especially in asthma in which the attacks are brought on by proximity to horses) or hay fever.

It has been suggested that, in cases necessitating second injections of antitoxin, a small amount, 1 c.c. or less, be given as a test dose to be followed by the necessary therapeutic dose twenty-four hours later.

It is important to be sure that the antitoxin is not injected into the vein because of the fact that anaphylactic symptoms are produced as much more readily by intravenous injections. This can be avoided by preliminary aspiration just before injection to see that blood does not enter the syringe.

Rosenau and Anderson have demonstrated the presence of anaphylactin in the blood of men who have been injected with antitoxic horse serum.

It has been suggested that by passive sensitization of guinea-pigs with patient's serum, we can ascertain whether or not there is any danger in second injections of serum.

It has also been suggested that a cutaneous test, similar to a v. Pirquet tuberculin test, be made with horse serum to find out whether or not hypersusceptibility to horse serum be present.

PART THREE—SPECIAL.

CHAPTER XXIV.

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 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.

Characteristics of the Organism.

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. "Droplet 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 immun-

ity. 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 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). The opsonin content of the serum in diphtheria is below normal at the onset of the disease. As the symptoms subside and the membrane disappears, the opsonic index rises considerably, returning to normal in from two to nine days.

Injection of dead diphtheria bacilli in suitable numbers into rabbits is followed by a rise in the opsonic index. Injection of dead diphtheria bacilli may prove of service in ridding the throats of bacillus-carriers of bacilli (Tunnicliff).

Recent experiments have substantiated the ideas of Behring that bacteriolysins are of little importance in immunity in diphtheria.

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 serotherapy 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.

Serotherapy. Experimentally, it is possible to vaccinate against diphtheria by the inoculation of dead diphtheria bacilli, or extracts of agar cultures (Lipstein, also Bandi and Gagnoni), but the conditions hardly warrant the use of this method for protecting man. Extracts of the organisms may be mixed with antitoxin and injected for protection. This is the so-called serovaccination.

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 should be remembered, however, that the dangers of anaphylactic symptoms are much increased by intravenous injection.

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 in which the patients 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 favorable 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 pseudodiphtheria 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 Microorganism. 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 new-born infants 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 to 1000 corrosive sublimate and in thirty minutes by the same solution to which 0.5 per cent. hydrochloric acid had been added.

Infection Atria and Conditions which Favor Infection. Tetanus is conspicuously a wound infection and that it develops so frequently from wounds which are contaminated with earth is readily understood 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 contami-

nated 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.

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 dia-

phragm, 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 (Wassermann'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 probability 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.

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. Ac-

Varieties of Tetanus.

quired 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 XXII, Part II. Its administration as early as possible after symptoms have appeared is demanded. After symptoms have existed for more than thirty hours Behring main-

tains 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 acomplish this object, or to attempt 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.

According to Anderson, the prophylactic dose of tetanus antitoxin standardized according to the official standard adopted by the United States Public Health and Marine-Hospital Service is 1,500 units. As a curative it should be given in doses of from 3,000 to 20,000 units, repeated during the course of the disease.

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

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.

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.

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.

According 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 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 hemo-

Ferments.

lytic agent (pyocyanolysin) of an alkaline nature, which resists boiling and is not a true toxin, since 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.

Toxin and Antitoxin.

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 bactericidal and opsonic is obtained. On the other hand, if an older toxin-containing bouillon culture be used, the serum is opsonic, bactericidal and antitoxic. The serum which is bactericidal and opsonic 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 cannot 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 mul-

tiples 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 of susceptible individuals, 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. Repeated use of this serum has been observed to result in sensitization of the patient to horse serum. Dunbar recommends in these cases that a very dilute solution be used.

Inhalations of increasing amounts of pollen, beginning with very minute quantities, has also been tried with the idea of active immunization.

It is probable that further study of hay fever as a phenomenon of anaphylaxis will result in the explanation of points concerning the disease which are not yet clear.

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 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 colubridinæ, among which is the American coral snake, possess immovable fangs.

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.

The hemolysin and neurotoxin, and perhaps other cytolysins of venom, consist of amboceptors

Amboceptors and Complement.

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 II, Chapter XVI. 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 administered not later than a few hours after the bite has occurred. Noguchi found in relation to antivenin for the rattlesnake that the quantity of 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

CHAPTER XXV.

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[1] (anti-infectious), 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 increased in bactericidal and opsonic power.

I. TYPHOID FEVER.

Eberth first saw *Bacillus typhosus* in microscopic preparations of the mesenteric lymph glands and spleen of a typhoid patient, 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

1. This has not been established in regard to Malta fever.

to twelve flagella situated at the ends 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 "intestinal group" of organisms, certain members of which are so similar that they can be differentiated only by means of special cultures, animal experiments, or the agglutinating, opsonic and bactericidal action of specific immune serums.[2]

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 excretions of the patients and carriers. 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

Viability and Resistance.

2. Of this group the bacilli 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.

80 days when dried on clothing, and for 3 months in typhoid feces. When in water or moist earth which contain many saprophytes its life is shortened. It survives drying for many months, although direct sunlight kills in the course of a few hours.

Endotoxin.

That the typhoid bacillus secretes a soluble toxin, has not been satisfactorily demonstrated. It contains, however, 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 and food 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 small 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.

The importance of the so-called bacillus-carriers as a source of epidemics of typhoid has been recently emphasized by a great many observers.

Park estimates that from 2 to 5 per cent. of all people who recover from typhoid, continue to excrete typhoid bacilli by way of the urinary or alimentary tracts. Park also estimates that one out of every 500 adults who have never had typhoid, harbor typhoid bacilli. The bile is generally regarded as the medium in which the bacilli perpetuate themselves in the case of the carriers.

By whatever means an apidemic 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 epidemics in military camps and elsewhere. Typhoid bacilli have been cultivated from flies which were taken from the vicinity of infected material.

The Infection Atrium.

The bacilli 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.

Quickly 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 probably all 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 cause agglutination. 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 develop on the plates may be identified by the usual bacteriologic methods, or the agglutination test may be performed with a known anti-typhoid serum. After from the tenth to the fourteenth day the organisms can rarely be cultivated from the blood; the bactericidal substances and phagocytic power 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 tem-

perature 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 non-pathogenic quantities of the bacilli into the cir-

culation. 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. According to Dreschfeld's figures 0.7 per cent. of individuals are affected twice. 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 immunity is created.

Acquired immunity is characterized by an increase of the bactericidal amboceptors, opsonins, agglutinins and typhoid precipitins in the serum. It has been shown that recovery is accompanied by an increase in concentration of antibodies. Bactericidal amboceptors reach a concentration two or three times that of normal serum and then return gradually to normal, reaching a normal concentration in a few months. Opsonins increase in concentration as do bactericidal amboceptors, but remain high for many months. Stone believes that this increased power of phagocytosis constitutes

the most important factor in the immunity resulting from an attack.

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 conclusions 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.

Hexamethylenamin.

Hexamethylenamin may be of value in causing the disappearance of bacilli from the urine, 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.

The typhoid carrier remains one of the difficult problems of prophylaxis. That carriers may be rid of bacilli by inoculation with dead bacilli, has not been satisfactorily demonstrated except in some instances. Systematic detection and treatment of these carriers is hard to carry out.

Serotherapy 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 Bristish 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 standardization. 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 estimating the number of bacilli in a cubic centimeter of the vaccine. The purity is insured by bacteriologic tests, and for preservation phenol or liquor cresolis compositus is added.

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 antibodies without producing any severe constitutional reaction." The inoculations increase the bactericidal, opsonic 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 "opsonins." 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," according to Wright, lasts for from one to several days and corresponds to a period of increased susceptibility. Russell and others have not observed this 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 varies from moderate to severe, but is 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

General Reaction.

next day his temperature comes down to normal, and he feels comparatively well except in respect to pain at the seat of inoculation." Of 5,473 soldiers vaccinated against typhoid, twenty-one took the disease and two died. In 6,610 soldiers under similar conditions who were not vaccinated, there were 187 cases and twenty-six deaths (Leishmann). The method of vaccination used by Russell and his associates in the U. S. Army is similar to that of Wright, but three injections ten days apart are given, the first of 500 million, the second and third of one billion. No bad results have occurred in 8,510 cases, and the results have been satisfactory, not a single case of typhoid occurring in any one whose vaccination was completed. Among the unprotected in the army 200 cases developed in the same period of time.

The vaccine was prepared as follows: A nonvirulent strain of the bacillus is grown on agar, slanted in flasks for twenty-four hours. The growth is then emulsified in salt solution and standardized to contain 1 billion bacilli to 1 c.c.

The vaccine is then sterilized by heating to 56° C. for one hour; 0.25 per cent. tricresol is added as a preservative and the sterility tested by aerobic and anaerobic cultures. The harmlessness is proved by inoculation into guinea-pigs and mice.

Conditions for Vaccination.

The adoption of antityphoid inoculation or vaccination under certain conditions appears to be warranted. Typhoid never has been a world pest; but in the presence of epidemics in densely populated districts, the method may well be considered. The question is a pertinent one also for those cities in which typhoid is so extensive as to be called endemic. It has a distinct field in the protection

of troops in time of war, when it is difficult to observe other prophylactic measures, and should recommend itself to physicians and nurses during epidemics.

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.

Serotherapy.

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 patients, 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.

MacFadyen 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 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 ani-

mals. The extract is administered by mouth. Jez justifies this method, from the fact that the lymphoid organs have been shown to form typhoid antibodies (Wassermann). From the clinics of Eichorst and 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 and is expensive. The suggestion of 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 has not had practical trial. Of a similar nature is the suggestion of Richardson, that the filtrates of typhoid cultures be injected. Richardson reports unsatisfactory results with various preparations of typhoid bacilli, including the non-toxic split products of Vaughan.

Anders has concluded from his results following the injections of killed typhoid bacilli, that the procedure is of value only in cases of relapse and in bacillus-carriers in order to rid the person of the bacilli. Doses of from 25 to 50 million bacilli were used and the injections were repeated every three days.

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 to 5,000 or higher, and from that time sinks gradually. An agglutinating

power of 1 to 160 has often been found at eight months, and of 1 to 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 les 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 Scholtmüller 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 in high dilution by antityphoid serum. Since

then, similar cases have been reported and two types of the paratyphiod bacillus have been recognized (Schottmüller). Bacilli of Group B cause first an acid reaction in milk which changes to a permanently alkaline reaction in about ten days, whereas those of Group A cause permanent acidity (Kayser). They resemble the typhoid bacillus morphologically, but culturally are more closely related 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.

Epidemiology. 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 fifty cases in East Holstein probably caused by the milk of two cows. Shortly after the epidemic began, the cows died and paratyphoid

bacilli were cultivated from the muscles, 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, opsonic 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 amebic 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-

Summer Diarrheas.

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.

The organisms of Shiga and Flexner differ in their actions on the sugars mannite and maltose (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. phenol in 30 minutes, 5 per cent. phenol 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 ill 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, Neisser 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 in typhoid. Water infection seems certain, and indirect transmission is accomplished 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.

The subject of acquired immunity to dysentery is hardly on a satisfactory basis. The serum of convalescents shows a distinct bactericidal and opsonic 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 and opsonins, 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

curative; the discharges rapidly decrease in number and the course of the disease is shortened. In the Rockefeller Institute for Medical Research anti-dysentery 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, putrid decomposition of the latter being 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 extremis*. 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 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 extremis* 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

Bacillus coli, 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.

Agglutinins.

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, the discoverer of 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 pathogenic powers which also exist normally in the in-

testines 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 to which the colon bacillus or *Bacillus* [*lactis*] *aerogenes* has been added, 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 v. 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 comma bacillus 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.

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 v. 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.

Virulence for Man.

The cultivation of the colon bacillus from the blood and organs of man at autopsy has not the

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.

Post-mortem 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 fifty-eight of sixty 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 organisms cultivated from the urine is noted in these cases. Davis and others have described cases of urinary infections due to *B. coli* differing from the usual type in that the growth on various media is less luxuriant and milk is not coagulated.

Davis has found that the serum of patients with such infections may be high in opsonic power and low in bacteriolytic, or *vice versa.*

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, opsonins and agglutinins.

Agglutination.

Not all strains of the colon bacillus are identical 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).

Specific Therapy.

Vaccine therapy has been successfully applied to many of these colon bacillus infections. The autogenous organism should always be used owing to variation in the bacilli, especially in infections of the urinary tract.

VI. CHOLERA.

In 1883 Koch discovered the *Vibrio choleræ* 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 situated 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 ob-

tained 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 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.

Identification.

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

Resistance.

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 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.

Epidemics.

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 il-

lustrating 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 epidemics 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 ex-

amination 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 subcutaneously (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, opsonins, agglutinins and specific precipitins in the serum.

Amako finds that opsonin, bactericidin and agglutinin develop with the course of the disease.

The length of the negative phase varies with the severity of the symptoms. The fulminating cases have a short negative phase ending in death. The antibodies reach their height during convalescence, the bacteriolysins usually developing most rapidly. In most cases the three antibody curves run parallel, but the bacteriolysins may be much more highly developed than the opsonins.

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 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 immunization for protective inoculation, using killed bacteria which have been saturated with the specific amboceptors.

Serotherapy and Agglutination.

Serotherapy 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.

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. Certain 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 Ghon); 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 thorouhgly 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 acetic 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, squirrels, 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. Guinea-pigs and rats 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 Ghon). 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. Cultures 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 and Squirrels.

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 believed that transmission from rat to rat may occur through the eating of plague cadavers. Experiments are also reported showing that fleas from plague-stricken rats will infect healthy rats, guinea-pigs and monkeys by biting them. The work of the Indian Plague Commission demonstrated that the usual means of transmission from rat to rat and from rat to man is by means of fleas. Monkeys, which are readily infected if put in the same room with infected rats, remain well if protected from fleas. It has been repeatedly demonstrated that fleas from rats and squirrels will feed on man.

In California, squirrels infected with plague are an important source of infection in man. Transmission from rats to squirrels and from squirrels to rats by means of fleas has been demonstrated by McCoy, and McCoy and Wherry report a case of transmission from squirrels to man, probably through fleas.

Flies, as well as fleas, may distribute the bacilli from rats or the infected excretions of man mechanically.

When plague invades a new country it commonly makes its first appearance in coast cities. Pre-

sumably 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.

Epidemics of plague lack the explosive-like suddenness 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 dissemina-

tion of the organisms. 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 who 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. In Cali-

fornia, the work of extermination of squirrels, rats and fleas has been carried on extensively by the U. S. Public Health and Marine-Hospital Service.

Vaccines.

The vaccine of Haffkine has been used extensively in India. The Indian plague commission found that the incidence of disease and the mortality 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 and 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 antiplague serum. Shiga reported good results by the use of the combined method in the epidemic in Kobe.

The immunity which is produced by protective 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. The influence of opsonins is essential for experimental phagocytosis, and is an important factor in the mechanism of immunity. Antiplague serum contains also complement-deviation antibodies and precipitins. The immunity which follows infection is of long duration.

Serotherapy 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 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 24 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 16 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.

According to Kolle and Krumbein antiplague serum should be tested as to concentration of all of

the various antibodies in order to obtain a correct idea of its value.

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. Diseases in which acquired immunity is not due to increased bactericidal power of the serum, 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 resistant 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, occur-

ring 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 (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 v. Emmerich and Löwe is not of established value.

Serotherapy 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 treatment, 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, also occurs among British troops at Gibraltar, and cases have been discovered

in the Caribbean Sea, Porto Rico, in Hongkong, Manila, and in India. 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. The incubation period is about fifteen days.

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. They disappear in about two years after recovery (Birt and Lamb).

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 with pure cultures have occurred through small wounds resulting in typical attacks of Malta fever (Birt and Lamb). The disease is not transmitted from person to person.

Up to the present time the monkey is the only animal known with susceptibility to artificial infection, although the organism has a certain virulence for rabbits and guinea-pigs on intraperitoneal or intracerebral injection (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.

CHAPTER XXVI.

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 the infections (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.[1] The serums of immunized animals may or may not be protective for other animals. Those organisms which cause systemic infection give rise to 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

1. This point is difficult of determination when an organism has little or no pathogenicity for animals (influenza, gonococcus, bacillus of Ducrey, etc.).

pulmonitis: *Diplococcus pneumoniæ, Streptococcus pyogenes, Staphylococcus pyogenes,* bacillus of Friedlander (*B. pneumoniæ*), *B. influenzæ, B. pestis, B. diphtheriæ, B. typhosus, B. coli communis, B. tuberculosis* and *Micrococcus catarrhalis.* The organisms of tuberculosis, actinomycosis, 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 Pneumonia.

Friedländer (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 pneumococcus, yet the cultures which he obtained somewhat later showed the characteristics of the organism now known as the bacillus of Friedländer. 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 and Rosenow); 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 G. F. Ruediger found a surprising number of pneumococci in normal throats, whereas previous work had shown them to be 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 embedded 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 55 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.

Virulence.

Rosenow and others have shown that virulent pneumococci are much less susceptible to phagocytosis than are non-virulent strains.

By autolysis the substance on which the non-susceptibility of virulent pneumococci to phagocytosis depends, can be removed and in this manner virulent pneumococci rendered readily phagocytable. Furthermore, by treating avirulent strains of organisms with the autolytic products of virulent organisms, the readily phagocytable non-virulent pneumococci can be rendered less susceptible to phagocytosis. This substance, which can be extracted from pneumococci and on which the resistance to phagocytosis depends, Rosenow has called "virulin." Virulin resists boiling for two minutes and is insoluble in alcohol and ether.

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 diphtheric enteritis, a condition which may be followed by pneumococcus peritonitis or general infection.

Entrance into 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 pneumococcus infections or of non-infectious diseases. In order to show that micro-organisms may be carried into the parenchyma 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 Infections.

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 organisms 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 phagocytes 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 con-

ditions 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 established for the study of acute respiratory diseases 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 disease. 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 patients should be isolated, 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 pneumococ-

cus 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 a matter of much discussion.

Neufeld and Haendel believe that the antibodies in pneumonia are inactive until a certain concentration is reached and that when a high enough development of antibodies occurs, there is a sudden neutralization of toxins with destruction of pneumococci leading to the crisis. These authors find that the serum of patients, who have passed the crisis, is protective for mice against many fatal doses of pneumococci. Other observers have failed to find any evidence that the crisis is due to antibody production. 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. The opsonic power of the serum in pneumonia is decreased in the early stages of the disease and reaches its height at the crisis and the following few days.

Serotherapy and Agglutination.

Some of the serums which have been prepared have been used therapeutically in man, but the results have not been sufficiently satisfactory to put

them on a good basis, although some favorable reports have been given.

Serum of Roemer.

The serum of Roemer 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. According to Neufeld and Haendel the disadvantage of the various antiserums lies in the fact that they cannot readily be given in large enough doses to be effective.

Agglutination.

The serum of pneumonia 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 agglutinable 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.

Vaccine Treatment.

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 doses. Rosenow has been able to separate, by a process of autolysis, the more toxic part of the pneumococcus, and the bodies of the organisms. By the use of the non-toxic part as antigen, a more rapid development of antibodies can be obtained, experimentally, than can be accomplished with whole pneumococci; Rosenow has made this fact the basis of treatment of pneumonia by means of injection of the autolyzed bodies of pneumococci and has obtained encouraging results. It is hoped that this procedure will be a valuable aid in hastening the crisis.

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, and muscular and subcutaneous abscesses arise.

Rosenow has isolated from endocarditis cases pneumococci differing from the usual type in that they grow in clumps and adhere more or less closely to the surface of blood-agar slants. They grow in chains and produce less green on blood-agar plates than do pneumococci of the usual type. In animal inoculation a tendency to localize on endothelial surfaces is shown.

Mode of Infection.

Other infections by the pneumococcus occur independent of the existence of pneumonia. Such conditions are alveolar abscesses, conjunctivitis, dacryocystitis, 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 much 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.

Serpiginous ulcer of the eye, a progressive phagedenic process in the cornea, is usually caused by the pneumococcus, 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* 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. Lying 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 millionth part of a cubic centimeter 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 from 0.01 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 de-

pends 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 fact is that virulent strains, when grown in serum and ascitic fluid, produce a substance which kills leucocytes and inhibits phagocytosis. This may explain the fail-

ure of leucocytes to take up virulent organisms, whereas non-virulent strains are readily phagocytized. Von 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). Von 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, 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.

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 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.

Erysipelas.

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. It is mostly of 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 in the form 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.

Meningitis. Primary streptococcus meningitis is rare or of doubtful occurrence. It frequently is secondary to otitis media, to injuries, and has been noted following tonsillitis, facial erysipelas, pneumonia, endocarditis and as part of a pyemic process.

Enteritis. Streptococci are at times the cause of enteritis in children, the inflammation often being membranous and accompanied by desquamation of the epithelium and by hemorrhages. It is not infre-

quently 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 conditions 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. Von 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.

Endocarditis. The endocarditis caused by streptococci usually 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, how-

ever. 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 micro-organisms 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. Tunnicliff found that the opsonic indices for *M. rheumaticus* (Beattie, Poynton and Paine) for *Streptococcus viridans* from the throat of a patient with rheumatism and for *Streptococcus pyogenes* follow the same course during attacks of rheumatism. In cases with joint symptoms and high temperature the index was subnormal and with improvement in clinical symptoms it

rose above normal. The indices for *Staphylococcus aureus,* pneumococcus and a strain of *Streptococcus viridans* from a normal throat did not show a variation from the normal. Agglutinins common to *M. rheumaticus* and streptococci were found to follow the same course as the opsonic index. Immunization of rabbits with *M. rheumaticus* resulted in the formation of opsonins for both this organism and *Streptococcus pyogenes.* These findings would indicate that streptococci play an essential part in acute articular rheumatism. The fact remains, however, that streptococci cannot 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, Gordon 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 diphtheric 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.

Gabritschewski and other Russians have reported satisfactory results following prophylactic vaccination against scarlet fever by means of killed streptococci from scarlet fever patients. They advance this experiment as an argument in favor of the streptococcus as the sole cause of scarlet fever. The results, however, have not yet been confirmed by other observers.

At present 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. Von 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 v. 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 clinical 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 streptococcus 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 bac-

teria 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 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 leucocytosis 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.

Weaver, Tunnicliff and Boughton have shown that the defense of the body against streptococci depends on the power of phagocytosis on the part of the leucocytes as well as the opsonin, hence the estimation of the resisting power of the body must be measured by the bactericidal power of whole blood.

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.

Immunization of Animals.

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.

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 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

Unity or Multiplicity of Streptococci.

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

Scarlet Fever. 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 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 v. Lingelsheim. It was heat-resistant (62° to 65° C.), and was not destroyed by dilute acids and alkalies (cited by Lingelsheim).

Stimulation of Phagocytosis.

Vaccine Therapy.

The prophylactic injection of killed streptococci in scarlet fever has been mentioned.

Reports regarding the curative injection of streptococci have been conflicting. It has been found that the use of galactose as a means of killing the streptococci results in a better preservation of antigenic properties than does the use of heat.

Weaver concludes that the use of therapeutic injections of galactose-killed streptococci is of value only in subacute and chronic streptococcus infections.

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 stain-

ing 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). Albumin 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 hemo-

lytic 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.[2] 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

2. Leucocidin and staphylolysin will not yield antitoxins when their activity has been destroyed by heat.

destructive action of the toxin on the leucocytes is indicated by the failure of this reduction when the toxin is mixed with the cells.

Toxic Filtrates. 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.

Endotoxin. 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 depends on its ability to resist the antibacterial activities of the body (phagocytic and digestive power of the leucocytes and opsonins). The part played by the leucocidin in this resistance is not definitely known.

Varieties of Staphylococcus. 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 *Staphylococcus albus* differs from the *Staphylococcus aureus* only in its inability to form pigment, and it cannot be made to acquire this property. Pigment is formed most 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 from thirteen to sixteen hours are required (Ottavino). 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 from 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 (v. 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.

Susceptibility of Animals.

Rabbits and mice are the most susceptible animals. The susceptibility of man is much greater. 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, v. Lingelsheim reduced the fatal dose for rabbits from 5 c.c. of a 24-hour broth culture, 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. They are found almost constantly in the lesions of impetigo and often in pure culture. They have been much vaunted as a cause of

Skin.

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.

Septicemia.

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

Serous Surfaces and Bones.

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 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.[3] They

Leucocytes in Natural Immunity.

3. Phagocytosis of staphylococci was first observed by Kirch in 1889.

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.

Bactericidal Action of Leucocytes and Leucocytic Exudates.

Experiments which were begun by van der Velde in 1894 demonstrate the bactericidal action of leucocytic 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.[4]

One may immunize either with living or killed cultures or with culture filtrates. 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, from 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.

4. Their experiments in protecting and curing other animals with antistaphylococcus serum represent the first attempt made in the direction of passive immunization.

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 many other chronic staphylococcus infections. 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 instances it has been noted that improvement and recovery go hand in hand with an increase in the opsonins. As in streptococcus infections the total resisting power of the body depends on the variation in the capability of the leucocytes to take up and digest cocci as well as the opsonic action of the serum.

"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 from 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 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 aggluti-

nating 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 Ghon and H. Pfeiffer found it 81 times, and M. Neisser demonstrated it in 16 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 Ghon, Pfeiffer and Sederl, "*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° or 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 inflammatory 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 pro-

duced 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 complications. 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 in-

fected 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 pubproperties. Torrey has shown that by immunization of animals a serum may readily be produced which contains specific bacteriolysins, agglutinins, precipitins and complement deviation antibodies. Hamilton and others have found that in gonorrheal vulvovaginitis the opsonic index is low in early stages and in those in which recovery does not take place, and becomes higher with recovery.

Serotherapy and Vaccination.

Torrey in 1906 prepared an antigonococcus serum by immunization of rabbits. Since that time, a number of different serums have been pre-

pared from such animals as horses and rams. The reports concerning the value of antigonococcus serum have been as yet too varied to admit of a conclusion.

The subcutaneous injection of dead gonococci for curative purposes has been apparently of little value in acute urethral infections. In chronic infections of the urethra, prostate and seminal vesicles some satisfactory results have been obtained. Cole and Meakins, and Irons find that the vaccine treatment of gonorrheal arthritis is of value in lessening the pain and in shortening the course of the infection.

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 Ghon); 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.

Viability.

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 Ghon).

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 present the organism does not produce a soluble toxin, but possesses rather an endotoxin. 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 inflammation 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, or blood. The latter, according to Elser and Huntoon, is probably the usual route. It has been found repeatedly in the noses of those associated with patients with the disease; in such cases an acute rhinitis may be present without the subsequent development of meningitis. The clinical history shows 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

Infection Atria.

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

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. Normal

cerebrospinal fluid does not contain opsonin for meningococci.

Serum Therapy.

In 1906, antimeningitis serum was prepared in this country by Flexner and in Germany by Kolle and Wassermann and Jochmann. The report of Kolle and Wassermann was the first to appear. It was quickly followed by that of Jochmann and later by that of Flexner.

Flexner prepared his serum as follows: Horses were inoculated subcutaneously with one-fourth of a sheep's serum agar slant culture of meningococci which had been killed by heating to 60° C. for thirty minutes. The dose was doubled at each subsequent injection until an amount equal to four test-tube growths could be given at intervals of from five to seven days. Alternate injections of living organisms and autolyzate of organisms were then given at seven-day intervals, in increasing doses until large quantities were given. The serum prepared in this way contains bacteriolytic amboceptors, opsonins, agglutinins and complement-fixation antibodies. It has also an antitoxic action on toxic autolyzates of meningococci.

Standardization.

Nearly all the above-named antibodies have been used to estimate the therapeutic value of the serum. Jochmann, and Kolle and Wassermann estimated the strength of antimeningitis serum by the protection afforded mice and guinea-pigs against live meningococci. The method is unsatisfactory because of the difference of virulence of various strains of meningococci. Jobling concludes that the estimation of the dilution of the serum at which opsonic action is still present gives the best indication as to the value of the serum. "As a definite and suitable standard of strength

a minimum dilution activity of a 1 to 5,000 dilution of the antiserum is proposed."

Action and Uses of the Serum.

Flexner and Jobling conclude from a study of the spinal fluid, that the most important action of the serum depends on bacteriolysis and increased phagocytic action. They give the following instructions for the use of the serum:[5]

"The antiserum should be kept in a refrigerator until it is to be used, when it should be warmed to the body temperature before it is injected.

"The antiserum is to be introduced directly into the spinal canal after the withdrawal of cerebrospinal fluid by means of lumbar puncture.

"The quantity of antiserum to be used at a single injection should not exceed for the present 30 c.c. It is desirable, although it would not appear essential, to withdraw from the spinal canal at least as much fluid as the amount of antiserum to be injected. The injection should be made slowly and carefully to avoid the production of symptoms due to increased pressure. This precaution should be exercised especially where the quantity of cerebrospinal fluid withdrawn is less than the amount of antiserum to be injected.

"The injection of the antiserum should be repeated every twenty-four hours for three or four days or longer. Whether any advantage will be gained by more frequent or more numerous injections than here indicated a wider experience must decide. As much as 120 c.c. of the antiserum have been injected into the spinal canal in four days without causing unpleasant symptoms.

"The evidence indicates that the earlier in the course of the disease the injections are made the

5. Flexner, S., and Jobling, J. W.: Jour. Exp. Med., 1908, p. 190.

better the results. Hence, should the film preparation of the first fluid obtained by spinal puncture show Gram-negative diplococci, some of which are within leucocytes, an injection should be made immediately and without waiting for the results of culture tests. Should the diagnosis be left in doubt or the disease prove later to be of another nature than epidemic meningitis, no harm will be done by the injection of the antiserum.

"Although the best results have thus far been obtained where the antiserum has been injected early in the disease, yet the serum should be used in its later stages also until our knowledge governing the value of the serum becomes more precise. The indications at present are that it is useless to employ the serum in the very late stages of the disease in which chronic hydrocephalus is already developed."

Value of the Serum.

Flexner and Jobling conclude from an analysis of a large number of reports of the use of anti-meningitis serum, that the serum is of value in reducing the period of illness and diminishing the fatalities due to the disease. The figures of Dunn show that in the Boston Children's Hospital, the mortality before the use of the serum, from 69 to 80 per cent., was reduced to below 20 per cent. after the use of the serum.

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 a trail of lesser epidemics which have prevailed up to the present time.

Bacillus Influenza. 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 pigeon, favor its growth. It is a strong aerobe. 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 animals which are known not to be susceptible to influenza, it is concluded that they can not be identi-

cal 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 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 circulation probably is erroneous. That metastatic infection is possible, by way of the lymph or blood

channels, is shown by the occurrence of influenza 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. Influenza 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 diseases. 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 respitory 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 peritoneum, 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 compartive 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 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 present 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 (Kraefting). 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 aerogenes*). 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 virulence for animals, immunization and agglutination tests, too variable to serve as bases for classifica-

tion. 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.

CHAPTER XXVII.

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 the animals used 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 Cohnheim. 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.

Characteristics of the Bacillus.

The tubercle bacillus is an obligate aërobic parasite, has the form of a slender, non-flagellated rod, 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.

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. **Staining Properties.**

Much has demonstrated in such cases, however, the presence of granular forms of tubercle bacilli which are not acid fast but which stain by a modification of Gram's method.

Ordinarily it is a difficult task to obtain the tubercle bacillus in pure culture, and the technic we need not consider here. 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 bovine serum 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. **Cultivation.**

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 24 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 extracted by dilute alkalies, contains a toxic nucleoalbumin. 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 bac-

terial 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.

In addition to the "old tuberculin," which has just been described, Koch has made several other

"TA," "TR" and "TO."

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 diagnostic value was equal to or exceeded that of tuberculin because of the longer duration of the reaction. However, in view of the fact 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 later 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 (*Bazillen-emulsion*).

Other Tuberculins. Preparations which in many respects are analogous to those of Koch have been made by various investigators; the tuberculocidin of Klebs, the tuberculins of de Schweinitz and Dorset and that of Denys, the two tubercule toxins 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 24 to 48 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. For the standardization of old tuberculin, the following method is used in the Royal Institute for Experimental Therapy, at Frankfort, Germany: Two series of guinea-pigs infected with a pure culture of tubercle bacilli are injected with decreasing doses of tuberculin. In one series a standard preparation of tuberculin is used; in the second series, the tuberculin to be tested is utilized. If the minimum fatal dose of the sample to be tested is the same as the standard, it is of official strength. If stronger than the standard it is diluted to the desired strength. If weaker it is concentrated by further evaporation.

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

Dried Sputum.

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.

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.

Bovine and Human Tuberculosis.

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 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 intestines 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 (Ravenel and others). In an examination of 436 cases of human tuberculosis, Park and Krumwiede found bacilli of the bovine type in fifty-two cases (11.9 per cent.). In persons over 16 years of age, constituting 297 of the 436 cases, one case of tuberculosis with bacilli of the bovine type was found (0.39 per cent.). In children between the ages of 5 and 16, nine out of fifty-four cases were due to the bovine type of bacilli (18.5 per cent.). In eighty-nine cases in which the patients were under 5 years of age, twenty-two due to the bovine type were found (nearly one-fourth). The cases showing bovine types of bacilli were mostly infections of the abdomen and glands of the neck. In no case of primary pulmonary infection were bacilli of the bovine type found.

Differences in the Bacilli.

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 phenolphthalein 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 justified in considering the meat and especially the milk of tuberculous cattle as the probable sources of infection in a limited number of cases.

Congenital uberculosis.

Comparatively few cases of undoubted congenital 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, Har-

bitz). The possibility of transmission of tuberculosis by means of tubercle bacilli in the spermatic fluid should also be considered. Although no proof of such infection exists, the presence of bacilli in the spermatic fluid has been demonstrated, particularly in men with tuberculous epididymitis.

Infection Atria.

Pulmonary tuberculosis is by far the most common form of the disease in man, and without doubt this usually 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 tissue 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 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 relocalization of a focus often causes a wide departgional 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 re-

gional 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 en-

dothelial 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 "epithelioid" cells.

Giant Cells.

Certain of the epithelioid 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 epithelioid

cells which have fused to form a multinuclear mass.

Retrogressive Changes.

Still more remote from the center of the tubercle, that is, surrounding the epithelioid 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 epithelioid and the surrounding lymphoid cells increase correspondingly, and new giant cells form at the periphery of the necrotic center, only to be included in 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

Formation of Fibrous Tissue.

Caseation, Calcification and Liquefaction.

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.

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 Infections.

Mixed infection, especially with the streptococcus, plays a very important part in the course of 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.

Disposal of Sputum.

The collection of infected sputum in properly constructed water-proof paper boxes, which, with their contents, should be burned daily, is the safest 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. phenol solution 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, produc-

ing 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 reac-

tion 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 present, however, we may not be justified in considering the subject a closed chapter.

It is the commonly accepted opinion that recovery from tuberculosis does not confer immunity to

Acquired Immunity.

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 tuberculosis, 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.

In the United States, Trudeau and de Schweinitz, and in Europe, Koch, Behring, Maragliano

Active Immunization.

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. Williams, Webb and Barber have successfully immunized animals by the injection of living virulent tubercle bacilli into the subcutaneous tissue. In these experiments, the immunization was begun with a single isolated tubercle bacillus as the first dose. The immunity was demonstrated by the fact that the animals were able to withstand the injection of many times the fatal dose of living tubercle bacilli. The indications are that in preparation of tubercle bacillus vaccines by heat, etc., the antigenic properties of the bacilli are unfavorably modified. 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 subcu-

taneously 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 1 mg. often causes an intense reaction. Weigert classifies the disturbance 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. It is often advisable in weak or young subjects to use 0.05 or 0.1 mg. Many authorities never exceed 0.1 mg. as an initial dose. If no rise in temperature is produced by this amount, a second injection of a larger quantity may be given after an interval of two or three days. Koch used as high as 10 mg. before concluding that the reaction is negative. Lowenstein and others recommend the cumulative action of three or four small doses of tuberculin (0.1 to 0.5 mg.) at intervals of three days. The advantage of this method is due to the fact that the diagnostic value of a reaction with a small dose of tuberculin exceeds the value of reactions with large doses. By this method, many patients are said to react with the first small dose while the cumulative action of subsequent doses results in a reaction in less susceptible individuals.

Theories of the Tuberculin Reaction.

Koch explained the tuberculin reaction by the harmful or necrotic effect on the leucocytes and other tissue cells. The substances formed by the breaking down of these cells give rise to the fever and other symptoms. In tuberculous tissues this effect of tuberculin is much more marked.

Babes supposed that the increased susceptibility of tuberculous persons was due to a summation of effects of the products of the tubercle bacilli in the tuberculous focus and the injected tuberculin. Von Pirquet and Schick explain the tuberculin reaction as a phenomenon of allergy. This explanation is the most satisfactory one. (See Allergy.)

Limitations in Diagnostic Use of Tuberculin.

In view of Naegeli's finding of tuberculosis, healed or active, in 97 per cent. of autopsies, the value of the tuberculin reaction would seem to be a relative one, and that the number of positive reactions obtained would depend on the amount of tuberculin used.

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 leprœ.* 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 ap-

parently indifferent substances as trypsin, peptone (albumose), sodium cinnamate 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."

Cutaneous Reaction of V. Pirquet.

Von Pirquet made use of the increased capability of the skin of tuberculous patients to react to tuberculin as a means of diagnosis of tuberculosis (see Anaphylaxis). The test is carried out as follows: The ventral surface of the forearm is cleansed with ether and two drops of old tuberculin are placed on the skin at points about 10 cm. apart. The skin underneath the tuberculin is then scarified over an area about the size of a pinhead, as for an ordinary small-pox vaccination. A small quantity of cotton is then placed over the scarifications until they are dry. A third scarification is made about 10 cm. from one of the first two and no tuberculin used. This is to be used as a control.

The ensuing reactions are described by v. Pirquet as follows:

1. Traumatic reaction: The vaccination and control sites show in a few minutes a small papule surrounded by a soft red areola which disappears in a few hours. There remains a small slightly raised pinhead-sized red spot which becomes covered with a crust. This is succeeded by pigmenta-

tion and then a gradual return to normal within a week or two.

2. Negative reactions show the same phenomena as the control site. The swelling lasts only twenty-four hours, and the areola is under 5 mm. in diameter.

3. The positive reaction: (a) Incubation period, which lasts from three to twenty-four hours. In most cases the reaction is fully developed in twenty-four hours. Those developing later than twenty-four hours v. Pirquet calls "torpid." These torpid reactions occur more frequently in older than in young children and in clinically unsuspected cases. It occurs in manifest tuberculosis only exceptionally.

(b) Development: The inflammatory reaction begins usually with a slightly raised areolar reddening which spreads from the scarification site and increases rapidly in diameter and height. The diameter of the papule is on an average about 1 cm. but may reach 3 cm. Small vesicles may form on the surface of the papule. The color differs with the normal coloring of the skin; usually it is of a deep red color. Very pale papules sometimes develop in cases of fatal tuberculosis (cachectic reaction). The border of the papule is sometimes sharp, sometimes irregular and at times small papules may be found surrounding it.

(c) Retrogression. The maximum development is usually reached in forty-eight hours and after this the swelling declines and the red color changes to a violet, then to a yellow color and finally becomes brown. The swelling disappears usually in from five to eight days and the pigmentation

in a few weeks. The best time for a single observation is 48 hours after the vaccination.

(d) Secondary reaction: In cases giving a negative reaction a second test may be followed by a positive reaction. In this case, the first vaccination site may show a slight reddening.

Value of the Reactio

The cutaneous reaction is a very delicate one and many cases of healed tuberculosis give a positive reaction. Since most adults, according to autopsy findings, have healed foci of tuberculosis the reaction as an indication of active lesions is of value only in children below the age of puberty.

Various modifications of the v. Pirquet reaction have been devised but cannot be discussed here.

The Ophthalmic Reaction.

Calmette proposed the instillation of tuberculin into the conjunctival sac as a diagnostic procedure in tuberculosis. In negative cases this is followed by only a slight reddening. In positive reactions a more or less severe conjunctivitis follows. The reaction has not become popular owing to the possibility of danger to the eye.

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 patients. 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 0.1 to 0.05 milligrams 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.

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.

It is probable that the therapeutic value of tuberculin depends on the utilization of the subcutaneous and other body cells as a source of antibodies. The formation of these antibodies follows the injection of tuberculin, whereas in the tuber-

culous process only the tissues directly involved are stimulated to antibody production. Koch published favorable results from the use of tuberculin 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.

Treatment with TR and "New Tuberculin."

Following the work of Wright and Douglas, the opsonic index has been used as a guide to the injection of preparations of tubercle bacilli. By the concentration of opsonins the state of immunity can be gaged and the dosage thus regulated as to time and amount. By means of this proce-

dure much valuable information has been obtained in regard to the avoidance of injections during the "negative phase" and the regulation of the size of the doses.

It is still a disputed question as to whether the condition of the patient as an indication for tuberculin injection can best be judged by the clinical symptoms or by the estimation of the opsonic index. There is no question, however, but that in suitable cases the proper application of vaccine treatment in tuberculosis is a valuable therapeutic aid.

Serum of Maragliano.

Maragliano publishes the following conclusions: (1) "It is possible to produce a specific (serum) therapy for tuberculosis; (2) It is possible to immunize the animal organism against tuberculosis as is done in other infectious diseases, and 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 antibody 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 1 c.c. 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 that 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 TO TUBERCULOSIS.

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 eliminated 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.*

Sheep.

The disease in mice is caused by a diphtheria-like organism called *Bacillus pseudotuberculosis murium* and is pathogenic especially for the gray mouse.

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 other lands only when intercourse was established. 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 nonmotile, has about the dimensions of the tubercle bacillus, the same staining reactions, and fre-

quently 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.

Duval has recently succeeded in cultivating the leprosy bacillus on media prepared as follows:

Cultivation.

The rind was carefully removed from the fruit portion of fully matured green bananas, every precaution to avoid contamination being used, and large blocks of the fruit, after slanting one surface with a sharp knife, were introduced into suitable sterile glass cylinders provided at the bottom with cotton plugs saturated in sterile distilled water. These plugs served not only as support for the banana, but as a source of constant supply of moisture. Sterile 1 per cent. solutions of tryptophan, cystein (made from protein), and leucin were next prepared and a portion of each poured on and allowed to saturate the banana. These solutions were used separately and in varying combinations, in order to determine on which the *B. leprœ* would grow best or grow at all. Both the banana and agar, which was saturated in a 1 per cent. solution of cystein, proved an excellent medium for the artificial cultivation of the leprosy bacilli when incubated at from 32 to 35 degrees C. The maximum growth occurred at a temperature of 32 degrees C. Light seems to favor the growth of *B. leprœ;* cultures kept in a glass incubator regulated at 32 degrees C. grew more rapidly than those in the dark chamber under similar conditions. Multiplication began early in the transplants and visible growth developed in the form of small, glistening, white colonies in from four to six weeks. Growth also occurred on the banana and agar when a solution of cystein and tryptophan had been added. The fact that growth occurred on the protein-cystein medium, and not on the others except in the presence of it, shows very conclusively that *B. leprœ* utilizes the end-products of digestion and not the products of cell metabolism. At least it is reasonable to assume that this is the case, if deductions may be drawn from these experiments. Multiplication

in vitro of an acid-fast organism was obtained from the transplanted leprosy tissue on the above mentioned media from four cases of leprosy which corresponded in every essential to the leprosy bacillus. Not only did the leprosy bacilli develop in the original cultures but they continued to grow in subcultures. That the artificial growth is *B. lepræ* there can be no doubt, as the morphologic and cultural features and the animal tests have clearly proved.

Lugai has shown that the leprosy bacillus is pathogenic for Japanese dancing mice. The bacilli not only multiply at the site of inoculation but become disseminated throughout the body, producing lesions having the typical characters of leprous lesions in man. Nicolli is said to have produced leprous nodules in monkeys by inoculating them with diseased tissue, and finally Duval has produced typical leprosy of the tubercular type in the monkey (*Macacus rhesus*), by means of pure cultures of leprosy bacillus.

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 occurrence, 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 until recently 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 suspectibility 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, 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 anti-leprosy 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.

Preparation of Mallein.

The method by which the mallein of Roux and 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. In experimental work with glanders extreme care is necessary as infection occurs very easily. Glanders has been transmitted to animals by rubbing bacilli on the intact skin. 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 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.

Agglutination of glanders bacilli takes place in high dilution with the serum of horses affected with glanders. An agglutination with serum in

1 to 500 dilution is a valuable aid to diagnosis. Normal horse serum is said to agglutinate glanders bacilli at times in 1 to 250 dilution. Agglutination in dilution of 1 to 5,000 and 1 to 10,000 has been observed. By active immunization of animals an agglutinating serum may be obtained, and such a serum may be used for the diagnosis of glanders bacilli. Precipitins are also formed.

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 maliein.

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).

IV. RHINOSCLEROMA.

(See page 572.)

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 somewhat characteristic sulphur-yellow macroscopic granules in the pus of the actinomycotic abscesses, and which give to the organism the name of "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.

Resistance. 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.

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 causing 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 occa-

sionally been found in diseased conditions of the tonsils and pharynx.

VIII. OIDIOMYCOSIS.

"Blastomycetic" Dermatitis.

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 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, Hyde and Montgomery, Ricketts and others. In many instances the specific fungi have been cultivated.

Systemic Oidiomycosis.

Further investigations by Rixford and Gilchrist, Busse, Curtis, Hyde and Montgomery and others have brought to light the existence of systemic infections by fungi which are identical with those found in blastomycetic dermatitis, and cases in which the disease primarily was limited to the skin have gone on to generalized infection. The converse is also true, that infections which primarily are systemic, or rather pulmonary, give rise to secondary invasion of the skin in a large percentage of the cases. Busse and Curtis both described infections with these organisms as *Saccharomycosis hominis,* on account of the fermentative powers of the organisms concerned. *Saccharomycosis hominis,* blastomycetic dermatitis and generalized blastomycosis are identical processes pathologically which have as their cause a group of fungi, the individual strains of which may show considerable differences. A similar disease which

has been observed in South American States and in California was formerly considered as a protozoic infection, but Ophüls and Moffitt have shown that this disease also is caused by a fungus which has many points of similarity with the organisms of local and systemic blastomycosis.

The number of observed cases of systemic blastomycosis has increased greatly of late. Twenty-four have been reported, and of these 18 or 19 are known to have proved fatal; three appear to have recovered spontaneously or under treatment, especially with potassium iodid or copper sulphate (Bevan). That the disease has often been passed over for systemic tuberculosis seems very probable (one such case is known), and that it is much more common than usually supposed is indicated by the recognition of five cases in the wards of the Cook County Hospital (Chicago) by Stober and others from June until January of 1907.

Nature of Fungi.

In blastomycetic dermatitis and systemic blastomycosis, the fungi proliferate in the tissue by budding, and are found chiefly in the intra-epithelial and subcutaneous abscesses, and in the granulation tissue, nodules and abscesses of internal organs. Their appearance in culture media and their 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 the term blastomyces is too narrow. He applies the name of Oidiomycosis to

the disease. 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). This feature is so constant that it would seem to constitute a specific difference between these organisms and those found in blastomycosis. There are reasons for believing, however, that endospore formation is a facultative property of at least some of the organisms of blastomycosis (LeCount and Myers), and if this proves to be true, the two groups are brought very close together biologically as well as morphologically. Ophüls calls this parasite *Oidium coccidiodes,* agreeing with Ricketts as to the generic character of the group, and the corresponding disease bears the name of coccidiodal granuloma.

Pathology.

The skin infection in both diseases 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. Microscopically, 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 organs, especially the lungs, spleen and kidneys, skin and joints, are the seats of abscesses and nodules which contain the parasites in immense numbers, and many giant cells

of the Langhans type. The lungs show lobular or more extensive consolidation.

The lymph glands show little involvement in blastomycosis; it is believed that metastases usually take place through the blood stream, which may depend on the large size of the organisms. On the other hand, there is marked lymphatic involvement in coccidioidal granuloma, and it is probable that the liberation of minute endospores favors lymphatic metastasis. Pathologically, the two diseases seem to be differentiated somewhat by the fact that coccidioidal granuloma presents a greater degree of necrosis and caseation than blastomycosis, and the lesions in the former bear a closer resemblance to tuberculosis than do those of blastomycosis (Hektoen). The differences, however, seem to be in degree rather than in kind, indicating a certain lack of correspondence in the pathogenic properties of the organisms concerned.

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 pulmonary tuberculosis. In extensive involvement of the lungs the organisms may be demonstrated in the sputum.

At present, little is known concerning immunity to these infections. Ricketts prepared a vaccine by disintegrating the organisms in a ball-mill, and in collaboration with Eggers found that the immunization of animals with the vaccine causes the formation of agglutinating or precipitating antibodies (from unpublished experiments). The practical value of the vaccine has not had a thorough trial. Christensen and Hektoen used it in two cases of systemic blastomycosis which, however, were so far advanced that no conclusions as to the value of the treatment could be drawn. Theoretically, the conditions would seem to be favorable for the vaccine treatment of blastomycosis, since the disease is of a chronic character and there is little opportunity for autoimmunization on account of the dense capsule which surrounds the organisms. By grinding the organisms up, their constituents may be injected in such condition that they are readily absorbed.

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 normal. 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.

CHAPTER XXVIII

GROUP V.

DISEASES DUE TO SPIRILLA.

I. RELAPSING FEVER.

The Organism.

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 *Spirillum obermeieri*[1] and are recognized as the cause of the disease. Novy describes two forms of the organism. The short forms vary from 7 to 9 microns, and are about 0.25 microns in width. The long forms vary from 16 to 19 microns. They result from processes of agglutination or multiplication. The organism is provided at one end with a long flagellum. The turns of the spirals of the short forms are two or three in number. The spirilla are very motile, and not only move from place to place but rotate on the long axis.

1. The spirillacæ, 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. According to Novy, the organism of relapsing fever has a rigid cell body with an end flagellum and would therefore be be classed as a spirillum.

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.

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. 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 spirochete does not appear in any of the excretions, unless these 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. Novy has found that the disease can be readily transmitted to white rats and mice; rabbits and guinea-pigs appear to be refractory. In mice, as in monkeys and man, relapses occur regularly. In rats, however, immunity is established after one attack. 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 is immune.

Immunity.

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 spirochetes are found within polymorphonuclear leucocytes.

Tictin also found the spirochetes 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 spirochætæ which again invade the body from the spleen.

According to Novy and Knapp, two distinct types of protecting substances develop during the course of the disease. They describe a germicidal substance which causes bacteriolysis both *in vitro* and as observed in Pfeiffer's phenomenon. In addition to this germicidal substance, they believe that a second protecting substance, which they term the immune body, is present. The existence

of the immune body is established by the fact that passive immunization can be accomplished by the use of serum having no germicidal action. Phagocytosis is concerned chiefly with organisms killed by the germicidal agent. Marked agglutination occurs with immune serum.

The immunity conferred by an attack of relapsing fever is probably of long duration. Other animals are also immune to a second infection.

Theory of Relapses.

The development of immune bodies which occurs with the first febrile period is insufficient to cause complete destruction of the spirilla. A few of these, aided possibly by their sheltered location in lymph spaces, survive and may become immunized to some extent against the antibodies. These organisms by multiplication institute a second febrile period which is followed by a higher development of immune bodies. Each relapse has the effect of heightening the immunization until complete destruction of the organisms occur.

Hereditary Immunity.

Hereditary immunity may result from intrauterine infection. Spirilla have been found in the heart's blood of the human fetus. Novy and Knapp describe the occurrence of both active hereditary immunity occurring in the young of infected rats and passive hereditary immunity occurring in the young of rats passively immunized.

It is evident from the work of Novy and Knapp that the chief difficulty in the production of a curative serum is that of the cultivation of the spirillum. It may be possible, however, to immunize larger animals with infected blood and thus obtain an efficient antiserum.

In addition to the European relapsing fever there are at least three recurrent fevers caused by varieties of spirilla distinct from one another in morphology and according to Kolle and Schatiloff in complement deviation. One of these three forms occurs in India. The other two are known as African tick fever. Of these two forms, that of West Africa was studied by Dutton and Todd. The other form is prevalent in East Africa and was studied by R. Koch. According to Koch, the ticks which carry the organisms also transmit them to the eggs, which in turn develop into ticks capable of infecting man. Koch found spirilla in only a part of the eggs of infected ticks.

II. SYPHILIS.

Historical Data.

It is impossible here to describe or even mention the many cocci, bacteria and protozoa (?) which have been brought into etiologic relationship with syphilis. Until recently, the bacillus of Lustgarten 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.

Spirocha~ta Pallida.

In 1905, Hoffman and Schaudinn discovered in the primary and secondary lesions of syphilis, a very delicate spirochete which they named *Spirochæta pallida* on account of the difficulty of staining it with anilin dyes.

The spirillum is of corkscrew-like form with from six to thirty turns. It is about ¼ micron in thickness, and from 4 to 26 microns in length. The turns are regular and deep in the middle and become less pronounced toward the ends. There is a fine flagellum at each end of the spirillum. When observed in serum by means of dark-field illumination, the organism exhibits marked motility. Movement may be observed both forward and backward; rotary and bending motion is also seen. Stained with Giemsa's eosinate of azur, the spirilla are stained a pale rose color. According to Schaudinn, division takes place longitudinally, and in this respect the spirochete resembles the trypanosomas. The systematic position of the organism is not yet certain. Cultivation has been reported by a number of workers. The cultures were not pure, however, and the spirochetes were non-virulent.

Anatomic Distribution.

The *Spirochæta pallida* has been found in the lesions of all stages of syphilis. These organisms are found in great abundance in the primary lesion and in the tissues of the infected regional lymph glands. They are easily detected in the tissues affected in secondary syphilis. Although found in the circulating blood, they occur only occasionally or in small numbers. In the organs affected by fetal syphilis, spirochetes are found in

great abundance. In tertiary syphilis the lesions contain only a few organisms. Those present are most numerous in the tissues surrounding the necrotic center.

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 animal 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. These observers 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). Spirochetes have been found in all the lesions of experimental syphilis in monkeys.

Syphilis in Other Animals.

Bertarelli first succeeded in producing experimental syphilitic keratitis in the rabbit and found associated with it the *Spirochæta pallida.* His work has been verified by various observers. Mühlens and others have been able to produce a primary lesion in the guinea-pig by material taken from syphilitic keratitis in the rabbit.

Spirochaeta Pallida as the Cause of Syphilis.

The fourth postulate of Koch, that of cultivation in pure culture and reproduction of the disease by means of such pure cultures, has not yet been carried out. The occurrence of the organism as described has, however, been such strong evidence that the *Spirochæta pallida* is accepted as the cause of syphilis.

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.

Virulence.

Clinical experience indicates that the virulence of the *Spirochæta pallida* 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 organism 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.

There is no reason to believe that natural immunity to syphilis exists in man. It was formerly believed that the fact that many prostitutes who were exposed to syphilis over a considerable length of time and who at no time showed active symptoms, were immune to the disease. The finding of positive Wassermann reactions in a large percentage of such individuals would indicate, however, that they did acquire syphilis. Through the application of the Wassermann test, it has also been shown that the laws of Colles and Profeta are also incorrect. The former states that the mother who gives birth to a syphilitic child without herself showing signs of the disease, is immune to syphilis. Knöpfelmacher and Lehndorf obtained positive Wassermann reactions in 56 per cent. of such mothers. Profeta's law states that a healthy child, born of a syphilitic mother, can suckle the mother without becoming infected. In this case many of the so-called healthy children have been found to be syphilitic, and others which were actually non-syphilitic have been observed to contract the disease from the mother.

Regarding second infections, experiments on apes have shown that second infections are readily produced at any time after the primary lesion has developed. Such infections are possible even after thorough courses of treatment terminating in recovery. These second infections differ from the first in that the incubation period is shorter and

the course of development and healing of the lesion is more rapid.

Apes with tertiary syphilis react (according to Finger) to inoculation with syphilitic material, with the formation of tertiary lesions.

Finger conceives of the process of immunity in syphilis as similar to the phenomenon of allergy of V. Pirquet. That is in a variation in the capability of reaction without the establishment of non-susceptibility. Second infections with syphilis have also been observed clinically.

Seroreaction and Serotherapy.

The serum reaction is discussed fully in the chapter on complement deviation.

The efficiency which is promised by the recent preparation of Ehrlich, known as salversan, leaves but little to be desired as a therapeutic agent. The lack of production of immunity also renders the possibility of a curative serum very doubtful.

III. FRAMBESIA.

Frambesia or yaws is a tropical disease found in both hemispheres. Castellani found a spirillum associated with the lesions which corresponds morphologically with the *Spirochæta pallida.* Owing to this similarity in the organisms, and to the fact that yaws resembles syphilis clinically, the two have been considered as different forms of the same disease. Castellani, however, finds that in the complement deviation reaction neither antigen nor antibody can be used interchangeably. He considers the two spirochetes as distinct from each other and names the spirochete of yaws, *Spirochæta pertenuis.* Transmission occurs by direct contact and probably also by means of flies.

IV. OTHER SPIROCHETES.

Among other pathogenic spirilla may be mentioned *Spirochæta anserina,* of the spirillosis of geese, *Spirochæta gallinarun,* causing a fatal disease of chickens and *S. Theileri,* found in a disease of cattle in Africa. The last two are transmitted by ticks.

CHAPTER XXIX.

GROUP VI.

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.

Segmentation of Asexual Cells. 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., from forty-six to forty-eight hours after the preceding one—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. From 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 Estivo-Autumnal Fever.

The parasite of æstivo-autumnal fever is from 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 from 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,[1] hence the bites of young mosquitos do not convey

1. This is questioned by Schaudinn.

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.

The æstivo-autumnal parasite apparently is more virulent than the tertian or quartan. Not 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 from two to four hours, the paroxysm is more intense but shorter than when division extends for from 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 Symptons 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

Fever and Schizogony.

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 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.[2]

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

2. 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

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 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 or-

gans 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. 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.

Cerebral and Intestinal Symptoms.

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 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 hemo-

"Black-Water Fever."

lysis is entirely obscure; the effect is that of an intense intoxication, in which the erythrocytes suffer primarily and chiefly. Craig warns against the administration of one large dose of quinin in the 24 hours in æstivo-autumnal fever lest pernicious symptoms develop.

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. It is not directly contagious. In order to become infected it is necessary, customarily, 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 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 other phenomena of malaria which were once very obscure have been cleared

Anopheles. up by the mosquito theory. 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 suddenly 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 0.5 liter of petroleum (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 the fact 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 serotherapy has been accomplished, and it is doubtful if any serum could equal quinin in efficacy.

MALARIA OF BIRDS.

Diseases considered to be true malaria also occur in birds.

Proteosome.

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. This 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.

Trypanosomas vary a great deal in size and morphology. Roughly, they are from one to five microns thick and from 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 of trypanosomes is nearly always longitudinal, rarely transverse. 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.

Koch and others have described sexual reproduction in the tsetse-fly.

Classification.

Koch divides the trypanosomes into two classes as to constancy in respect to: (1) morphology; (2) virulence; (3) host. This classification is best represented by the accompanying table from Nocht and Mayer.

PRINCIPAL TRYPANOSOMES ACCORDING TO NOCHT AND MAYER

Division According to R. Koch	Zoologic Name	Described by	Cause of Disease	Animal as Host	Geographic Distribution	Transmission
Group I — Constant as to Morphology, Virulence and Host.	*Tr. lewisi* (Kent, 1880)	Chaussat, 1850 Lewis, 1878	Usually causes no symptoms of disease.	Wild rats.	Universal.	Fleas and lice.
	Tr. theileri (Laveran, Bruce, 1902)	Theiler, 1902	Gall sickness.	Cows.	South Africa.	Hippobosca rufipes (?)
Group II — Inconstant as to Morphology, Virulence and Host.	*Tr. evansi* (Steel, 1885)	Evans, 1880	Surra.	Horses, cows, camels, dogs; less often other animals.	India, Indo-China, Philippines, North Africa.	*Tabanus tropicus* and *lincola* *stomoxys calcitrans* and *nigra.*
	Tr. brucei (Pimmer and Bradford, 1899)	Bruce, 1894	Nagana fly disease.	Most mammals, especially domestic.	Most of Africa.	*Glossina mors.* and *Fusca,* *Gloss. pallidipes.*
	Tr. equiperdum (Doflein, 1901)	Rouget, 1894 Schneider and Buffard, 1899	Dourine.	Horses.	Europe-Asia, North Africa, North America.	Through coitus.
	Tr. equinum (Voges, 1901)	Elmassian, 1901	Mal de caderas.	Horses.	South America.	*Mosca brava* *stomoxys calcitrans* and *nebulosa.*
	Tr. gambiensa (Dutton, 1902)	Dutton, 1901	Sleeping sickness.	Man.	Equatorial Africa.	*Glossina palpalis.*
	Tr. castellani (Kruse, 1903)	Castellani, 1903				
	Tr. ugandensa (Castellani, 1903)					
	Tr. dimorphon (Dutton and Todd, 1902)	Dutton and Todd, 1904	Trypanosomiasis of horses.	Horses.	Senegambia.	*Glossina palpalis,* *stomoxys?*

TRYPANOSOMIASIS IN MAN.

Trypanosomatic Fever.

Nepveu 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. nepveui*). Again in 1901 Forde discovered similar parasites in Western 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).

Occurrence. 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.

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 cerebrospinal fluid, but this occurred very rarely during life and at a late stage of the disease; such organ-

isms 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 cerebrospinal 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 very 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. Gray and Tullach have demonstrated the presence of trypanosomes in the alimentary canal of tsetse flies which were allowed to feed on the blood of patients with sleeping sickness.

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 trypanosomatic 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 trypanosomatic 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 trypanosomatic 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 trypanosomatic fever. After from 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 trypanosomatic 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. nepveui* 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 from 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 trypanosomatic 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 caderas* 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, 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 dependant 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 streams occurs periodically. With a paroxysm 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 caderas*). 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: from 1.4 to 3 microns in diameter, and from 10 to 25 microns in length, according to different observers. It is of lancet-form, possesses a finely 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 endomedium 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 con-

densation 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).

Nagana. *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 morphologically 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 1.5 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.

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.

Tsetse Fly. 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.

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 from 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 Caderas. *Trypanosoma equinum* (Voges) or *T. elmassianii* is the parasite found in *mal de caderas,* 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 caderas* 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. 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 a 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."

When a dose of trypanroth which is insufficient to cause complete disappearance of trypanosomes is given, the remaining organisms become immune to the further action of the drug. It is of importance, therefore, to give the largest dose which is non-toxic for the patient. At present, two new preparations of Ehrlich, trypanosan and agridinum, which are highly trypanocidal, are being tried in combination with arsenophenylglycin.

III. TEXAS FEVER.

Texas fever of cattle may be considered briefly as a well-established example of piroplasmosis.

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. Larvæ hatch from the eggs in from 3 to 4 weeks, and the former are again 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.

Ameba. 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 (*Amœba 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 from ten to fifteen months. A temperature of 50° C. kills the vegetable and encysted forms. Sunlight for three hours and the *x*-ray kill them readily in the vegetable 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. sodium hydrate (Frösch), and strong acids. They resist the action of 0.2 per cent. hydrochloric acid, i. e., the acidity of the stomach contents. Quinin (1/2500 of the hydrochlorate) is strongly germicidal for *Amœba 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. *Amœba 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 *Amœba 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, 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 organisms, 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

Pathogenicity.

account they held the amebæ to be the sole cause of the dysentery.

According to Schaudinn and Craig, amebœ are of two types. One of non-pathogenic character (*Entamœba coli*) found by Craig in 50 per cent. of normal stools; the other pathogenic (*Entamœba histolytica*).

Lesions.

According to Schaudinn, the two organisms differ in morphology and method of reproduction. Walker fails to confirm these observations.

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 un-

cooked 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 from 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 from 8 to 9 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 meas-

ures from 70 to 100 microns in lengths and from 50 to 70 in breadth. Proliferation is through simple division. Conjugation has been noted. Involution cysts are sypherical 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).

Two species are found in man. *Trichomonas vaginalis:* possesses three flagella and an undulating membrane, and is of large size (from 15 to 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 significance, 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 species of trichomonas occur in the intestines of different animals.

Other Flagellates. 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.

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 nu-

Life Cycles. cleated 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.

Results of Infection.

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.

Coccidium bigeminum, a coccidium in which the oöcyst divides into two spore-containing cysts, has been found in man several times.

X. KALA-AZAR.

Kala-azar, or febrile splenomegaly, is a tropical disease, especially of India and China, associated with great enlargement of the spleen, often of the liver, extreme cachexia and anemia. The disease was formerly looked on as a malarial cachexia.

Both Leischman and Donovan described bodies in stained preparations of splenic pulp and ascribed to them an etiological significance. These observations have been confirmed repeatedly. The bodies are small round mass of cytoplasm, which with the Romanowski stain is colorless. Two masses of chromatin, one much smaller than the other, take a purplish stain. The whole body is from 2 to 3 microns in diameter. Rogers succeeded in cultivating the organisms in blood slightly acidified with citric acid and incubated at 22° C. In this way a flagellated form was obtained which is similar to trypanosomes in morphology. The classification, however, is not yet certain. The organism is distributed throughout the body, but is most numerous in the spleen, bone marrow, and liver.

Patton succeeded in obtaining growth of the parasite in the stomach of the bed bug, and it seems probable that transmission occurs in this way. Rogers, acting on this supposition, was able to reduce the number of cases by ridding the houses of bed bugs.

CHAPTER XXX

GROUP VII

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.

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, and it is now generally conceded that the bodies

are specific for rabies, and of great diagnostic value. 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 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 ultramicroscopic 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.

Toxin.

The possibility that the organism secretes a soluble 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).

Street Virus and Fixed Virus.

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.

At this point it is called fixed virus (*virus fixé*), 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.

Although *virus fixé* 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 fixé* 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.

Incubation Period. 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.

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, from 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 substances mentioned 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 309).

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.

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.

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.

In communities in which hydrophobia is known to be endemic, in all cases of dog bite accompanied by penetration of the skin, the patient should receive 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 fixé* 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 désiccation 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.

According to Harvey and McKendrick, the degree of infectivity of dried rabic virus is a "function of the loss of weight in water caused by the drying."

Technic of Treatment.

As is well known, the Pasteur prophylactic treatment consists of the subcutaneous injection on successive days, of suitable quantities of *virus fixé,* prepared as described above, beginning with the cord which has been desiccated for fourteen days and gradually using fresher cords until virulent 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 708, 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 attenuation 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 Centanni); the use of fresh but very dilute virus (Högyes). Ferran, in Barcelona, inoculates man with the fresh unaltered *virus fixé,* and in nearly 2,000 cases but two cases of hydrophobia developed. This indicates clearly the low infectiousness of *virus fixé* for man.

The tendency at present is toward the use of fresh rabic virus for the prophylactic treatment of hydrophobia. This is the method of Högyes, and also of Ferran. Högyes' first injection consists of 3 c.c. of a 1 to 10,000 or 1 to 8,000 dilution of the fresh rabic cord, and gradually within the next fourteen days the concentration is increased until 1 c.c. of a dilution of 1 to 100 is given.

In order to obtain a basis of comparison for the different methods of treatment Harvey and McKendrick have proposed an arbitrary unit of standardization for rabic virus. For this purpose they agreed to consider that 0.2 c.c. of a 1 per cent. emulsion of the fresh *virus fixé* contains 1,000 units. From this it follows that 0.2 c.c. of a 1 to 1,000 emulsion would contain 100 units, and 0.2 c.c. of a 1 to 1,000 dilution, 10 units.

The first dose in the Högyes method according to this scale represents 150 units. Naturally those methods in which dried virus is used for at least part of the treatment can not be expressed in units until the infective value of the cords dried for different periods is determined. This was investi-

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

gated by Harvey and McKendrick and their conclusions were as follows: "(1) Emulsion of the nine-day cord is little if at all infective in a dose of 0.2 c.c. of a 1 in 5 emulsion. (2) Emulsion of five-day cord is infective in minimal time in a dose of 0.2 c.c. of a 1 in 100 emulsion, but become less so or not at all in a dose of 0.2 c.c. of

a 1 in 200 emulsion. (3) In the same way the M. I. D. (minimum infective dose) for an emulsion of three-day cord is 0.2 c.c. of a 1 in 200 emulsion. (4) The M. I. D. of two-day cord is not greater than 0.2 c.c. of a 1 in 1,000 emulsion and probably not less than 0.2 c.c. of a 1 in 2,000 emulsion. (5) The M. I. D. of one-day cord is not greater than 0.2 c.c. of a 1 in 4,000 emulsion and almost certainly not less than 0.2 c.c of a 1 in 8,000 emulsion (the lower accepted limit of fresh material). (6) Fresh material is infective (M. I. D.) in a dose of 0.2 c. c. of a 1 in 8,000 dilution and may be so in considerably higher dilutions even up to 1 in 40,000, but with such high dilutions the experimental errors become so great as to preclude any more exact fixation of the M. I. D."

Although these results are not mathematically exact, it is probable that they may be used as a working basis, and from them it is possible to calculate the number of units in a given amount of rabic cord dried for different periods. Harvey and McKendrick estimate that in both the Pasteurian method and that of Högyes little more than 25,000 units are administered during the course of treatment.

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 to 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.

Babés 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. YELLOW FEVER.

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). Finlay 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 coworkers 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, however, 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 a yellow-fever patient. 2. In order that the mos-

Important Facts Which Have Been Learned.

quito 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 from 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 *Stego-*

myia 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 which 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 Ringgold (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 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 Pre-

Time of Biting.

vention 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 susceptible 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.

An attack of yellow fever, whether experimental or natural, confers immunity of long or lasting du-

Immunity and Serum Properties.

ration. 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.

III. "SPOTTED FEVER" OF THE ROCKY MOUNTAIN STATES.

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 these localities as spotted fever, is encountered in the months of spring. 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."

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 piroplasma (*Piroplasma 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 ameboid 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.

After identifying the organism as a piroplasma and having in mind the part that ticks play in the transmission of Texas fever, and perhaps piroplasmosis 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 twenty-three 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.

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.

Infectiveness for Man and Other Animals.

McCalla and Brereton infected two individuals successively by the bite of a tick which they had removed from one of their patients.

Ricketts and his collaborators have shown that spotted fever can be reproduced with great constancy in the guinea-pig by the injection of infected blood or the organs or eggs of infected ticks. The symptoms of spotted fever in the guinea-pig appear after an incubation period of from two to five days. There is a sudden rise in temperature to 105° or 106° F., with a generalized roseolar eruption. Swelling and hemorrhage of the scrotum or vulva occurs. The symptoms are diagnostic when they occur typically. The monkey, rabbit, horse and at least five species of small wild animals have a greater or less degree of susceptibility.

Ricketts and King, working independently, were able to transmit spotted fever from diseased to normal guinea-pigs by allowing ticks which had fed on diseased pigs to bite normal pigs. Ricketts and Wilder were able to show that up to 50 per cent. of infected ticks transmitted the infection to their young. It was found that nymphs developing from infected larvæ were infectious for guinea-pigs, and that in a similar way adult ticks developing from nymphs were able to transmit spotted fever. Naturally infected ticks were discoveerd in

1907. In order to ascertain the probable source of the virus as occurring in the tick, Ricketts showed that ground squirrels, rock squirrels, chipmunks and ground-hogs were susceptible to the disease.

Maintenance.

Ricketts concludes as follows as to the maintenance of spotted fever: "In accordance with the results and deductions which have been outlined, it is conceived that spotted fever is maintained as follows: A certain percentage of the female ticks which have acquired the disease as a consequence of feeding on animals, the latter having been infected by other ticks, transmit the disease to their offspring through the egg. The new generation, during the process of feeding, transfer the virus to certain of the susceptible small wild animals (ground squirrels, rock squirrels, chipmunks, ground hogs, and perhaps others), and this may take place during either the larval, nymphal or adult stage, hence at various times of the year. During the infection of the wild animal it is required that hitherto normal ticks, either as larvæ, nymphs or adults, acquire the disease by feeding simultaneously with, or shortly after, the feeding of the infected ticks. Regardless of the tick's stage of development at the time it acquired the disease, the virus is retained into the adult period, and in certain of the females reaches the germ cells and again appears in the next generation. The infection of man is an unessential incident for maintenance, and depends on the occasional and accidental bite of the infected adult tick."

The virus of spotted fever is not filterable through Berkefeld candles. The eggs of infec-

Microbic Etiology.

tious ticks were found by Ricketts to contain a large number of small polar staining bacilli. These organisms were agglutinated in high dilution by the serum of spotted fever cases, but are also found in the eggs of non-virulent ticks. Attempts at cultivation by Ricketts and Heinemann have been negative.

Immunity.

No authoritative report of a second attack of spotted fever is on record. According to Ricketts and Gomez, an attack of spotted fever in the guinea-pig and monkey produces a strong active immunity of long duration. This immunity is characterized by the presence of protective antibodies in the serum which may be demonstrated by injecting mixtures of virus and immune serum. The concentration of the antibodies in the blood of the immune animal undergoes a decrease in the course of several weeks.

The female that has recovered from spotted fever transmits immunity to her young. The young are immune even when the female acquired her immunity several months before impregnation. The immunity of the young does not depend on the ingestion of milk from the immune mother. The character of the inherited immunity has not yet been determined, although it is presumptively a passive immunity that differs from the passive immunity conferred by the injection of immune serum by its longer duration. The long duration of the inherited immunity may depend on the longer time required for the elimination of large quantities of protective substances.

Passive immunity may be established in the healthy guinea-pig by the injection of blood or

serum from the immune guinea-pig. The immune defibrinated blood contains antibodies in such concentration that 0.1 c. c. often protects against 1 c. c. of third-day virus, representing anywhere from 30 to 100 minimum pathogenic doses. In other instances 0.3 or 0.4 c. c. of immune blood are required for this degree of protection. When 1 c. c. of strong immune blood is injected subcutaneously into healthy guinea-pigs, the passive immunity is still present in marked degree after twenty days; after thirty-eight days it is present only in such degree that a mild course of spotted fever results when virus is injected; after forty-five days it is no longer manifest. It is possible that passive immunity would not last so long if the immune blood is injected into a foreign species.

The guinea-pig may be protected against spotted fever following its inoculation with infected blood, provided the immune blood is administered on the second or third day after inoculation.

Prophylaxis and Serotherapy.

The work of Ricketts indicates that efforts at prophylaxis are to be directed toward the extermination of ticks and the wild animals which harbor them.

Serotherapy will probably depend on the cultivation of the microbic cause of the disease.

IV. TYPHUS FEVER.

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.

Mexican typhus, or tabardilli, resembles European typhus closely, but has a longer incubation period and reaches the crisis a few days before the European typhus does. There is less tendency to confluent eruption and hemorrhage.

Pathogenicity for Animals.

Nicolle and his associates were able to produce typhus in the chimpanzee by the injection of blood from European typhus patients and in a similar way in the Macacus monkey with blood from infected chimpanzees. Direct transmission from man to the macacus was not accomplished. Anderson and Goldberger were able to transmit tabardillo directly to the monkey by inoculations with the blood of typhus patients. Their results were confirmed by Ricketts and Wilder, who determined the following points: "1. Macacus rhesis can be infected with tabardillo invariably by the injection of virulent blood from man taken on the eighth to tenth day of fever. 2. Attempts to maintain typhus in the monkey by passage through other monkeys were unsuccessful. 3. Monkeys

may pass through an attack of typhus so mild that it can not be recognized clinically. Vaccination results."

Transmission.

Nicolle succeeded in transmitting the typhus fever of Tunis from chimpanzees to monkeys by means of the common body louse. Anderson and Goldberger and Ricketts and Wilder succeeded in producing tabardillo in the monkey through the bite of the louse. The lice were allowed to feed on the blood of patients with tabardillo and subsequently permitted to bite the monkeys. Ricketts and Wilder also demonstrated that infected lice transmitted the infection to their eggs, which gave rise to lice capable of infecting monkeys. Their observations of the spread of the disease render it reasonably certain that lice are the ordinary means of transmission. Studies on the bed bug and flea indicate that they play no part in the spread of this infection.

Microbic Etiology.

The virus of typhus fever is not filterable. Various organisms have been described in the blood. Ricketts and Wilder describe a small bacillus in stained blood preparations. The organism resembles the plague bacillus and those organisms described in spotted fever. The organism was also found in the intestinal tract of infected lice. Cultivation was unsuccessful. Further studies are necessary to establish the etiologic relationship to typhus.

Immunity.

The production of immunity through a light attack of typhus in the monkey has already been mentioned. The serum of convalescents is said to be curative in a moderate degree (Legrain).

V. DENGUE FEVER.

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.

"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. Eberle, in 1904, advanced the hypothesis that dengue is transmitted by a mosquito (*Culex fatigans*). He described a "plasmeba" in the blood of patients with the disease. Other observers have failed to find protozoa in the blood. Ashburn and Craig (1907) were able to transmit the disease to healthy men by injecting the blood of infected individuals. They were able to produce the disease by allowing mosquitoes, which had fed on dengue patients, to bite healthy men. Their studies also showed that the distribution of the *Culex fatagans* corresponded with that of dengue,

thus putting the hypothesis of Eberle on a firm basis.

Filterability of Virus.

Ashburn and Craig were unable to find the organism responsible for the disease, but demonstrated that the filtered blood of infected persons can produce the disease in healthy subjects. They were unable to cultivate or detect in other ways any micro-organisms.

According to Ashburn and Craig, an attack of dengue confers immunity. They were unable to produce a second attack by the injection of infectious blood in individuals who had recovered from the disease.

VI. ACUTE ARTICULAR RHEUMATISM.

(See p. 525.)

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.

Cytoryctes Variola, a Protozoon. (?)

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.

Work of Councilman and Others.

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 to 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, 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 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

Cytoplasmic Stages.

"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 intranuclear 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 a 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.

Cytorycetes 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. vacciniæ*, 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

Dissemination.

"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 con-

tact with patients, and the disease may be carried to others from the sickroom by a healthy person. 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.

We have nothing more than presumptive knowledge concerning the distribution of the virus in

Distribution of Virus in Body.

the body aside from its occurrence in the skin and mucous membranes. We may feel certain, however, 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.

Interesting matters of history are the facts that protective inoculation against smallpox was prac-

Discovery of Vaccination.

ticed 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).

Humanized Lymph. For at least half a century following Jenner's 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 from 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. Because 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 bac-

Effect on Contaminating Bacteria.

tericidal 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 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 (from 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.[1]

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

1. John F. Anderson "Federal Control of Vaccine Virus," Jour. of the Amer. Med. Assn., June 10, 1905.

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 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 obtained 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 incuba-

tion 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 serotherapy.

IX. SCARLET FEVER.

The rôle which the streptococcus plays in scarlet fever was considered on page 527.

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 anti-

septic 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. The prophylactic vaccination by means of streptococcus (Gabritchewsky) is deserving of further trial.

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 leucocyto-

sis (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. 527).

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.

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 epi-

demics 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 leucocytosis.

There is no serum therapy for measles. Weissbecker 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

Hemophilic Properties and Symbiosis.

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.

Pathogenicity. 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 intraperitoneally 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 whoop-

ing 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.

Bacillus of Bordet and Gengou.

In 1906 Bordet and Gengou isolated a bacillus from cases of whooping cough and gave the following reasons for believing that it was the specific etiologic factor in this disease: 1. The organism is found in overwhelming numbers during the early course of the disease and in almost pure culture. 2. The bacilli as antigen give a complement-fixation reaction with the serum of pertussis patients and this reaction does not occur with other bacteria associated with the disease.

The organism is a short, polar-staining ovoid resembling the influenza bacillus but slightly larger. Bordet and Gengou grew the organisms on a culture medium made up of a glycerin-potatoe-blood-agar mixture. On this medium the organism grows in the form of a delicate film made up of very small colonies and changes the medium to a dark brownish color.

Wollstein was able to confirm the finding of the bacillus in the early stages of pertussis, but failed to obtain the complement-deviation reaction. Agglutination was very irregular and no immune opsonins were found. The etiologic relationship of the organism to whooping cough is at present uncertain.

The organism is disseminated extensively by coughing, and the same is probably true of the es-

Contagiousness. sential 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.

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.

Serotherapy. Serotherapy 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 organ-

ism of other observers, hence, his antiserum can 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. Bordet found the serum of a horse immunized to his bacillus of questionable curative value.

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.

Diplococci in Mumps.

In 1893, Laveran and Catrin described a diplococcus obtained by aspiration of the exudate in the parotid gland. The organism was also isolated from the testicle in orchitis cases and from the circulating blood. Since that time, diplococci have been isolated from cases of mumps by a number of observers. In 1909, Herb isolated a diplococcus which she considers as probably identical with the organisms described by previous writers.

The organism was cultivated at autopsy from the lung, testicle, cerebrospinal fluid, bile, parotid gland and pericardial fluid. The coccus is a Gram-positive organism occurring mostly in pairs but also in short chains and small groups. It varies from 0.5 to 0.8 microns in diameter in twenty-four-hour cultures. It is non-motile, has no capsule, and forms no gas or indol. It grows slowly on ordinary media and much more rapidly on media containing saliva. The growth on saliva agar appears as pearly white pin-point discrete colonies.

Pathogenicity. The organism is fatal to white mice, white rats, guinea-pigs and rabbits when injected subcutaneously; when injected into Steno's duct in monkeys and dogs non-suppurative parotitis was produced and occasionally an orchitis. The evidence indicates strongly that the diplococcus described by Herb is the specific etiologic factor in mumps.

Immunity. One attack usually establishes protection. According to Herb, the opsonins would seem to play an important part in the protection of the body against mumps.

Patients should be isolated for three weeks from the time symptoms appear.

XIV. EPIDEMIC POLIOMYELITIS.

Epidemic poliomyelitis, or acute anterior myelitis, is an acute febrile disease of children and young adults accompanied by an acute inflammation of the cord and brain. Clinically, it is characterized by paralysis of various muscles, usually those of the extremities. The paralysis is very rapid in onset and varies in tendency to recovery and permanent disability.

The disease has been known for over half a century, but it has been recognized as an infectious disease for only a few years.

Micro-organisms.

Although various bacteria have been described in connection with the disease, there has been little reason for considering them other than mixed infections or contaminations.

Flexner and Lewis found that the virus of poliomyelitis is filterable and describe very minute bodies occurring in the infectious filtrate. The bodies can be stained with Loeffler's flagella stain and cause a cloudiness in culture media after suitable incubation. The transfer of a small amount of such cloudy media to a second clear media results again in cloudiness after incubation. The virus loses its virulence when heated to from 45° to 50° C. for half an hour, but resists freezing.

Experimental Poliomyelitis.

Landsteiner and Popper, in 1909, and Knöpfelmacher a little later, succeeded in producing poliomyelitis in monkeys by injection of emulsified cords of children dying of the disease. They were unable to infect second animals with material from the first. Later, in 1909, Flexner and Lewis were able to produce poliomyelitis in monkeys in a way similar to that described by Landsteiner and Popper, and succeeded in transmitting the disease from one monkey to another.

Infection may be produced in monkeys by injecting the virus into the brain, spinal canal subcutaneous tissue, peritoneal cavity or into the large nerves.

Distribution of the Virus

Experimental poliomyelitis can be produced by the injection of material from the blood at the beginning of the infection and by injection of

emulsions from the nasopharyngeal mucous membrane. The emulsions of central nervous tissues give the most constant results, while the emulsions of other organs such as liver, spleen and bone marrow have failed to give results. It is possible that infection takes place in a manner similar to the process in epidemic meningitis. That is by dissemination of droplets and particles from the nasopharyngeal membrane.

In a reinoculation of ten monkeys which had recovered from poliomyelitis, Flexner and Lewis observed no instances of second attacks. In normal monkeys 72 per cent. of those inoculated became infected and showed paralysis. Those which did not become paralyzed were suspected of mild attacks. It is possible that vaccination may be successful.

XV. NOMA.

Noma, or gangrenous stomatitis, is a somewhat rare disease of children whose resistance is lowered by the acute infectious diseases. Among these it is found most frequently associated with measles. Next to measles, it is oftenest found in typhoid, intermittent fever mercurialism, scarlet fever, pertussis, enteritis, variola and many other diseases.

Fusiform Bacilli and Spirilla.

Perthes, Seiffert, Matzenaur and others found associated with noma, fusiform bacilli and spirilla. These observations have since been confirmed by many others. The organisms are found in the necrotic tissues and especially at the line of advancing necrosis. Ellerman, in 1904, cultivated fusiform bacilli from a case of noma. Weaver and Tunnicliff obtained pure cultures of fusiform

bacilli from noma in 1905. The organism is an obligative anaerobe requiring a temperature of about 37° C. for growth. The presence of blood serum or ascites fluid is necessary for obtaining the best growth, but cultures can be obtained on glycerin agar. All cultures have a foul odor.

On ascites agar the bacilli occur as delicate pointed Gram-negative rods. In ascites broth the organisms are more slender, not so pointed, and tend to form chains. On solid media wavy filaments are found. In old cultures forms similar to the spirilla found in the tissues were found. Tunnicliff isolated from the gums of healthy subjects, fusiform bacilli which were apparently identical in cultural characteristics and morphology with those found in noma. In pure cultures grown several days spirilla were found rather constantly and it would seem as if the spirilla were simply a stage in the development of the fusiform bacilli.

The number of cases in which the bacilli and spirilla have been found associated with noma makes it strongly probable that they are the cause of the disease.

Fusiform bacilli and spirilla similar to those isolated from noma have been found in ulcero-membranous angina and stomatitis, in hospital gangrene and in fetid infections in various parts of the body.

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