

Vaccine and serum therapy : including also a study of infections, theories of immunity, specific diagnosis and chemotherapy / Edwin Henry Schorer.

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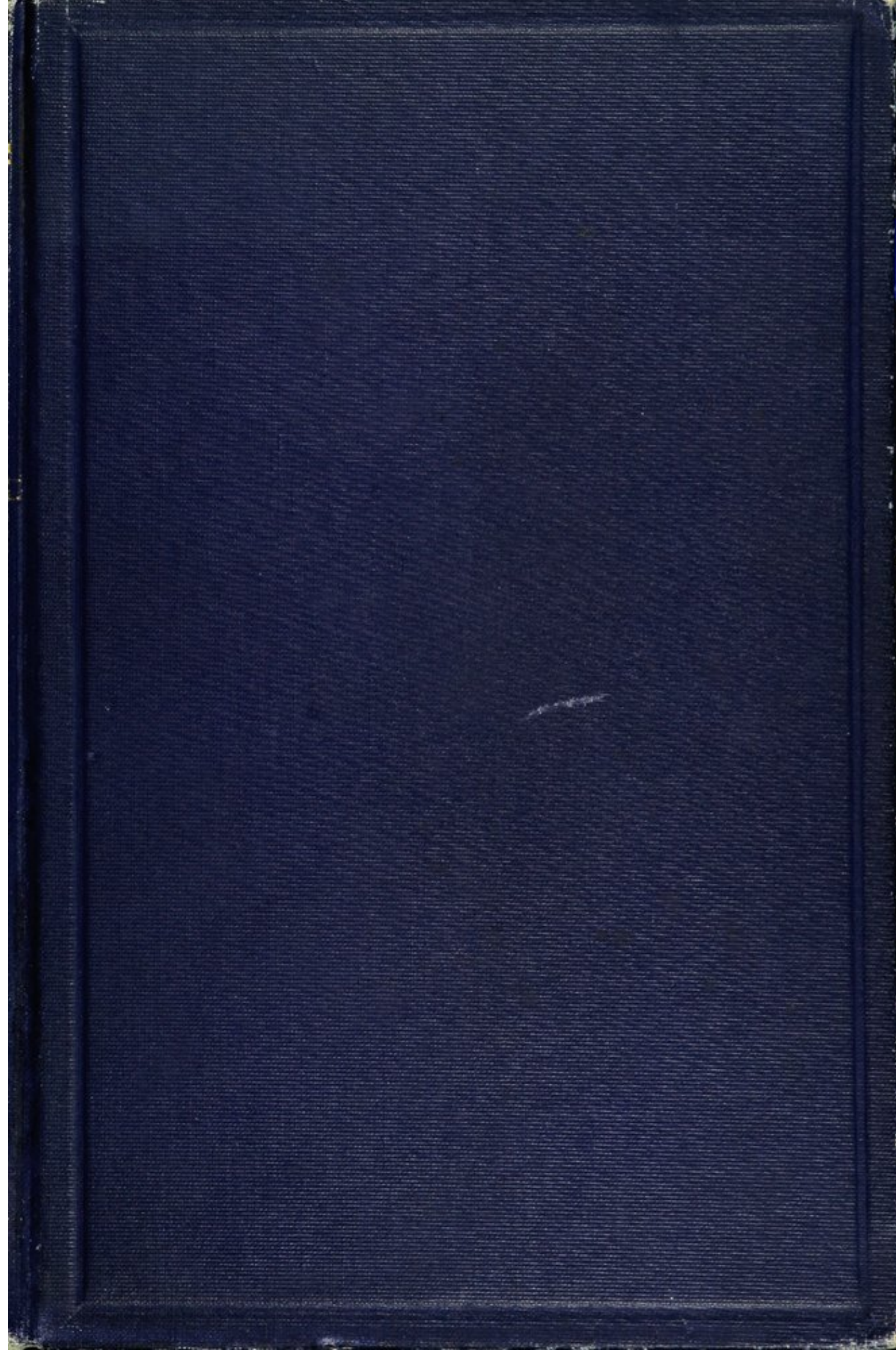
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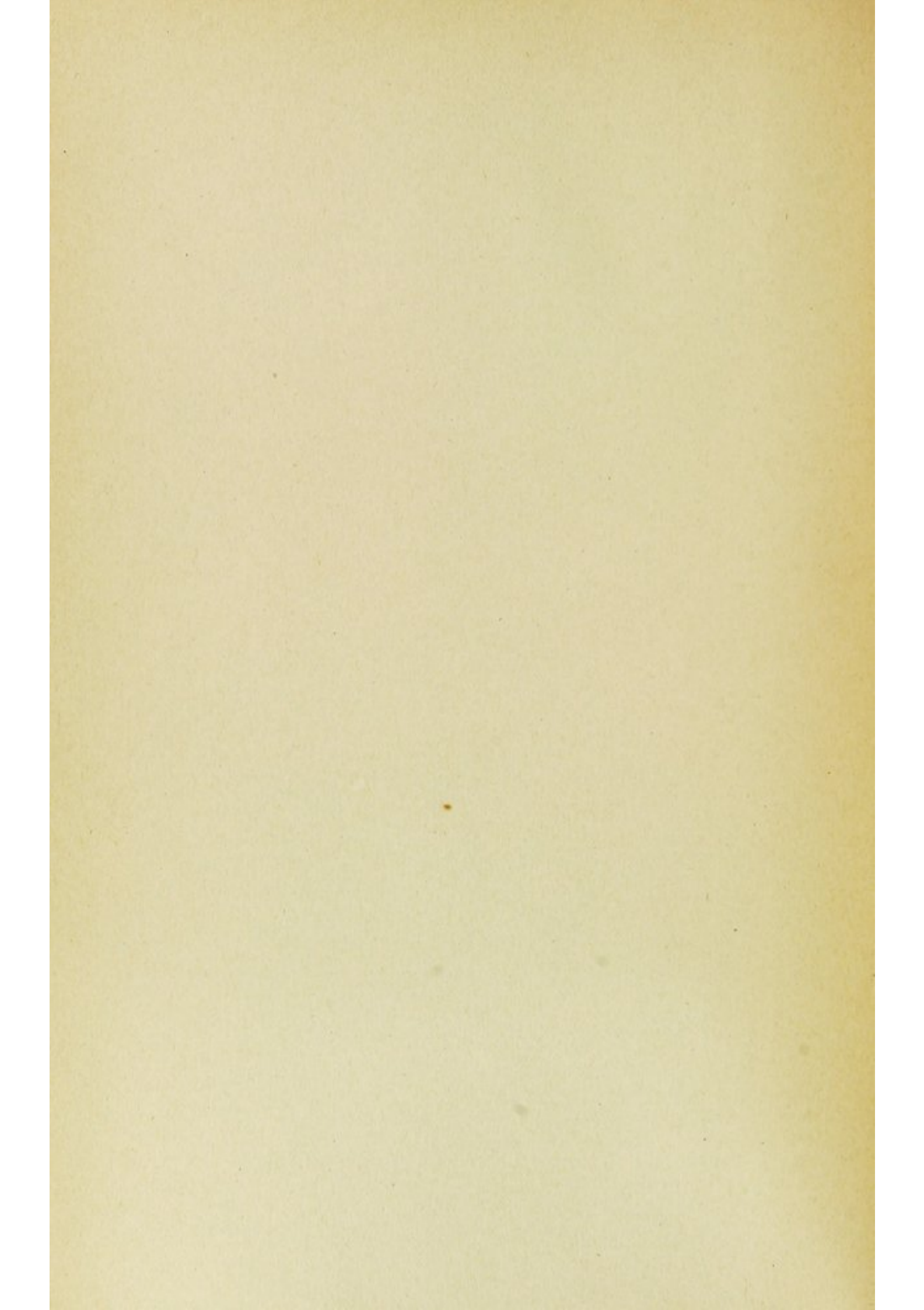
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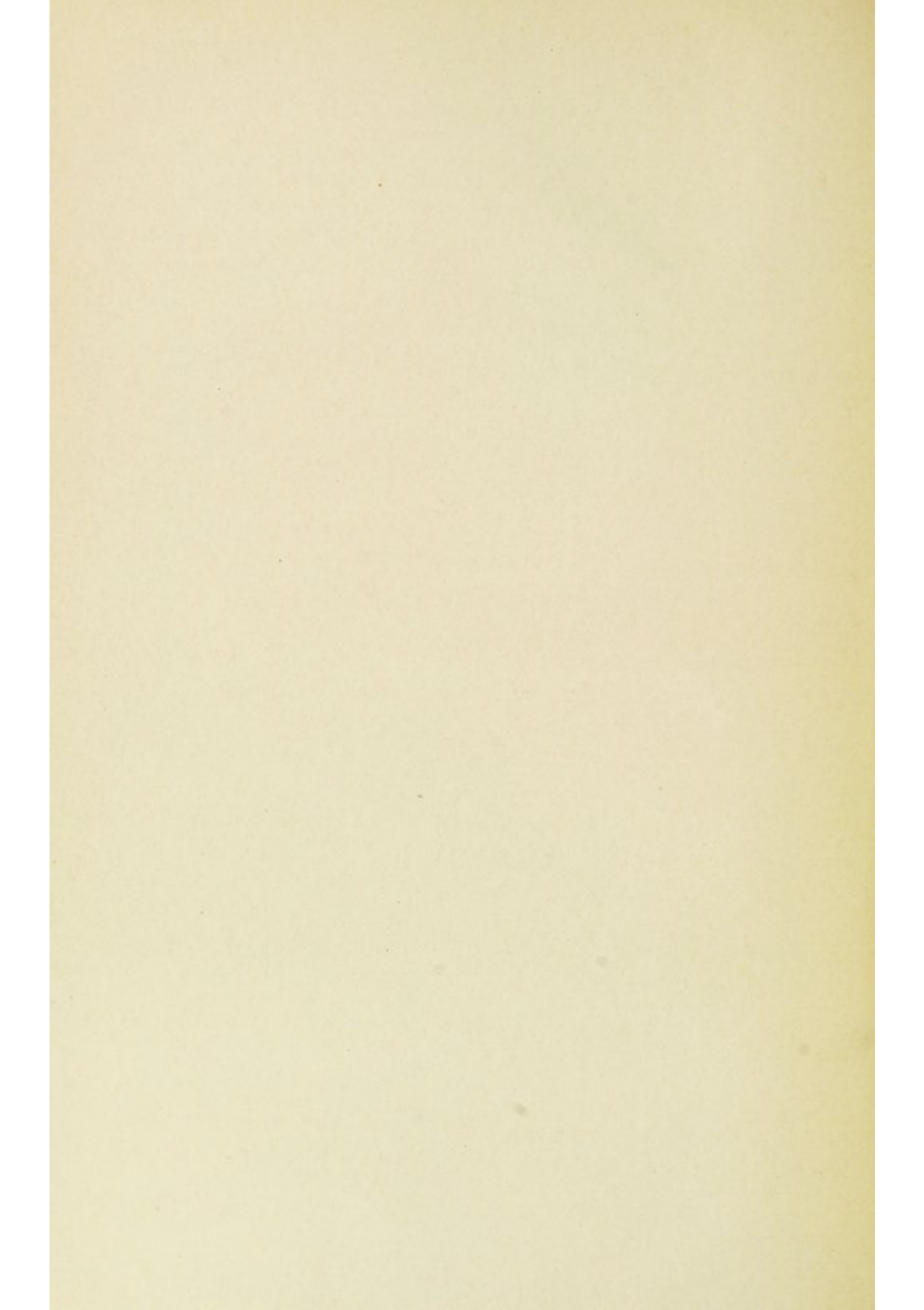
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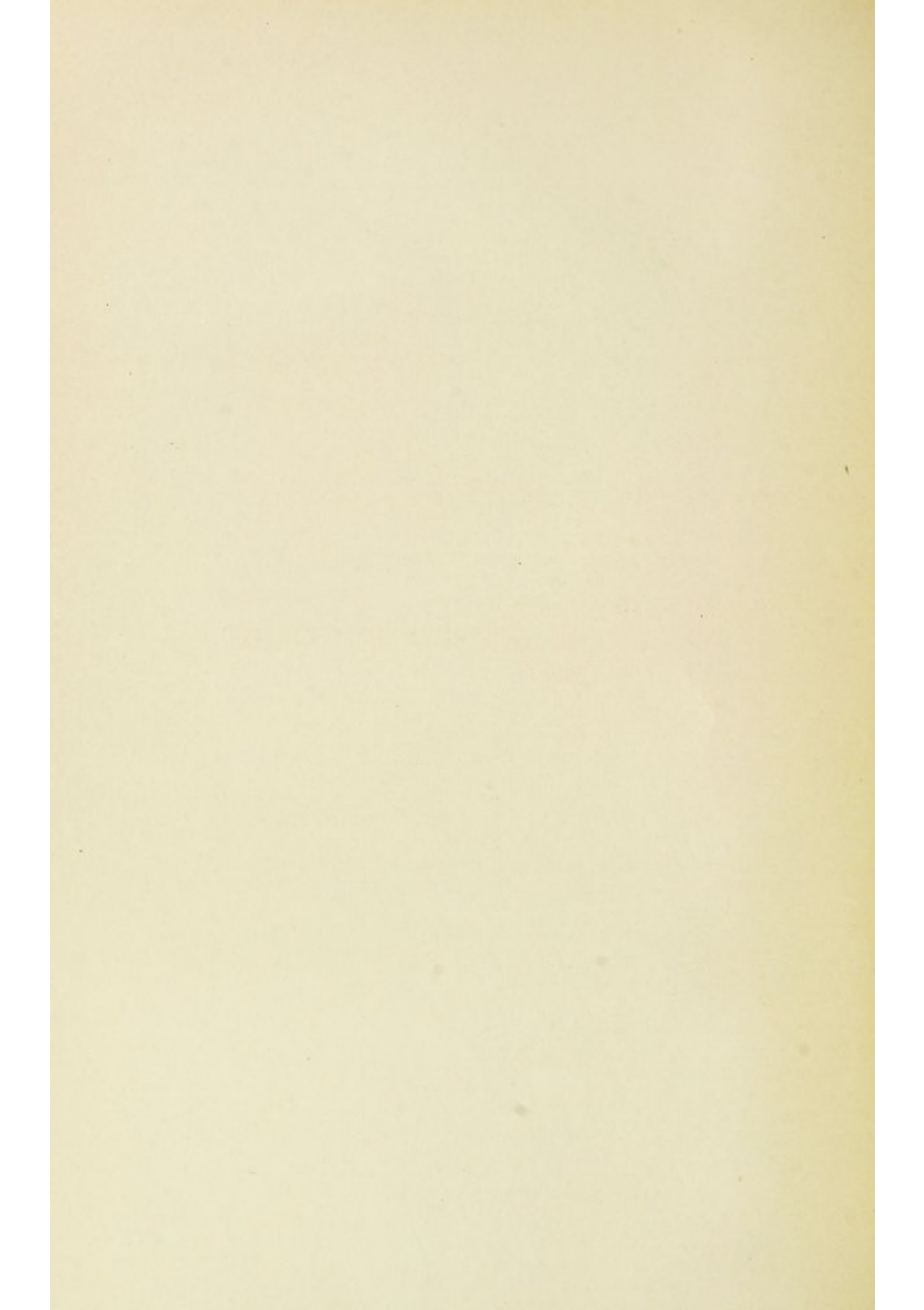
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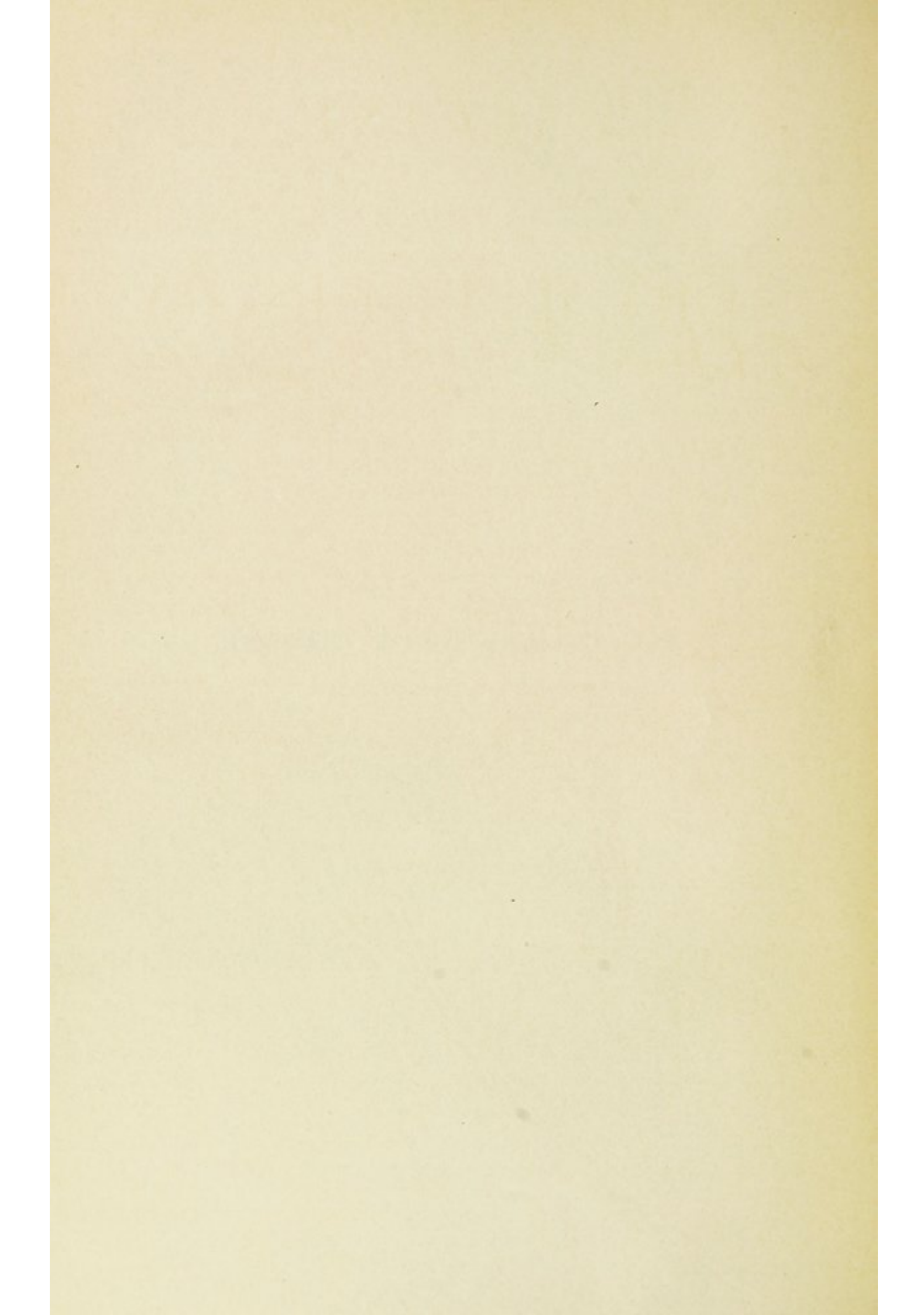
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VACCINE AND SERUM THERAPY



VACCINE

AND

SERUM THERAPY

*G. L. Hutchinson M.D.
Los Angeles Cal*

Dec 10 - 1914

INCLUDING ALSO A STUDY OF INFECTIONS, THEORIES
OF IMMUNITY, SPECIFIC DIAGNOSIS
AND CHEMOTHERAPY.

BY

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UNIVERSITY.

SECOND REVISED EDITION

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1913

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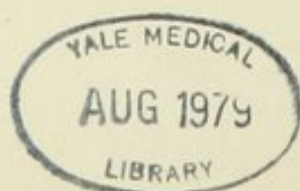
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PREFACE TO THE SECOND EDITION.

Since the publication of the first edition vaccine and serum therapy have changed considerably. Some of the theories and individual experiences have been substantiated by the experience of others while some have been refuted by further investigations. New studies have been of value in extending the scope and efficiency of vaccine and serum therapy so that now vaccines and sera are advocated for nearly all of the infections and infectious diseases. At the time the first edition was written, vaccine and serum therapy were just beginning to be used by the medical profession, few of the supply houses were preparing vaccines and the prices charged for vaccines were relatively high. Now laboratories especially those preparing vaccines have increased greatly in number, the indications and contraindications are better understood and vaccine and serum therapy seem fairly well established.

If vaccine and serum therapy amount to anything they do so because they are specific and for this reason their use must be based on the diagnosis of the specific etiological factors in the disease. There is no vaccine for boils, rheumatism, and so on, but there is specific treatment for infections with *Micrococcus pyogenes* and some of the other micro-organisms. To help those who may consult this work when resorting to vaccine and serum therapy an entire chapter on the general principles of specific diagnosis of the etiological factor has been added. Likewise in the last chapter differential diagnostic points have been given for infection with each of the organisms for which specific therapy is discussed. While much space is given to laboratory methods in diagnosis, differential clinical symptoms and signs are given so as to help in the laboratory diagnosis as well as to give the practitioner some assistance in making a specific diagnosis of the etiological factor when the clinical laboratory is not available. Because of the prominence of chemotherapy

and the value of chemicals in the treatment of certain infectious diseases, specific diagnosis and treatment of syphilis and malaria are added in the appendix.

Vaccine and serum therapy offer such great hopes as specific remedial agents that the profession must be informed about them. The physician should have enough knowledge to enable him to decide when vaccines or sera should be given and what results are to be expected from their use.

Before deciding on the value of the specific vaccine or serum it must be remembered that many infectious diseases are self-limited in their course. While there may be those who will differ to some extent from the methods of diagnosis and specific therapy given in this work, an effort has been made here to present the subject concisely and fairly and in such a manner as to be of the most assistance to the practitioner.

Kansas City, Missouri, May 1st, 1913.

PREFACE TO THE FIRST EDITION.

Treatment of diseases with medicines or by methods having a selective curative action has until recently been limited. With the establishment of the germ theory of certain diseases and the development of information concerning immunity, new methods of specific treatment were made possible and are now practiced under the name of serum and vaccine therapy. As might be expected, the medical profession has been much interested in these methods of treatment and applied them whenever possible. The development of vaccine and serum therapy has been slow, the methods have had to be revised and in some cases the results obtained have been found to be otherwise than was at first expected. Because of this much confusion has arisen. The practitioner has not been able to keep pace with the developments and literature on these subjects and finally has been forced to depend on the statements and recommendations coming from serum and vaccine laboratories, enthusiasts and even exploiters.

In this work an attempt has been made to state concisely and accurately the present knowledge concerning vaccines and immune sera. An effort has been made to establish theoretical and experimental evidence as well as clinical application of the specific treatment of bacterial diseases. To accomplish this, some space is given to infections in general, the theories of immunity, with especial emphasis on the opsonic theory of immunity, as well as the particular methods of vaccine and serum therapy.

Considerable space has been given to opsonins, the opsonic index and the importance of opsonins in health and disease. This has been done because since 1904 no subject has appeared more prominently or frequently in medical literature than that concerning opsonins, opsonic immunity, and bacterial vaccines. In the first presentations of discoveries on this form of immu-

nity and specific treatment of bacterial diseases great possibilities were promised. Methods which would, according to Wright, give uniform success in treatment of the large class of bacterial infections and diseases naturally received immediate and general attention by the medical world and were at once quite generally applied. This has been followed by much indiscriminate and unscientific use of these methods of specific treatment so that in the minds of many opsonins and vaccine therapy have gone into disrepute as did tuberculin nearly twenty years ago. We now know tuberculin has many applications of importance in the diagnosis and treatment of tuberculosis, though this knowledge has been gained some years later than it would have been had it not been for improper exploitation. In the hope of avoiding a repetition of such an effect as far as opsonin and bacterial vaccines are concerned, this subject is given considerable attention. The opsonic index technique which is given here is the one taught the writer by Dr. W. G. Ross, who was for two years a pupil of Sir A. E. Wright.

An attempt has been made to bring the subjects taken up as nearly up to date as is possible. It is hoped that this work may furnish to the medical student and practitioner information which may lead him to a better understanding of the nature of infections and the subjects of immunity, and active and passive immunization.

Columbia, Missouri, April, 1909.

CONTENTS.

CHAPTER I.

	PAGE
INFECTIONS	1-7
Course of infections	2-7

CHAPTER II.

IMMUNITY	8-30
Theories of immunity — Exhaustion theory; Noxious retention theory; Phagocytic theory of Metchnikoff; Chemical theory; Humeral theory; Ehrlich's side-chain theory; Opsonin or Bacteriotropin theory	10-30

CHAPTER III.

SPECIFIC DIAGNOSIS	31-93
Isolation and identification of etiological factors by direct microscopical examination, by cultural methods and characteristics, by serum reactions and by animal inoculations	35-39
Determination of etiological factor by the presence or absence of specific antibodies—Collection and preservation of serum to be tested; Agglutination, precipitation, bacteriolytic, fixation of complement and opsonic index determinations	39-82
Hypersusceptibility of allergy tests	82-85
Chemical and empirical tests—butyric acid, cobra venom, ammonium sulphate, albumin tests	85-89
Inclusion bodies	89
Filterable viruses	89-90
Leucocytes in the circulating blood in infections—Conclusions on tests for specific diagnosis	90-93

CHAPTER IV.

SPECIFIC THERAPY	94-137
Therapy dependent on specific antibodies	94-100
Vaccine Therapy—Preparation of bacterial vaccines, dosage, site and control of injections	100-112
Bacterial cell plasm and bacterial products	112-114
Immunization to infectious diseases of unknown etiology	114-115
Serum Therapy—Standardization of antisera, concentration and purification of serum; Indications for injection of serum, administration of antisera, untoward effects of serum administration (Serum disease)	115-134

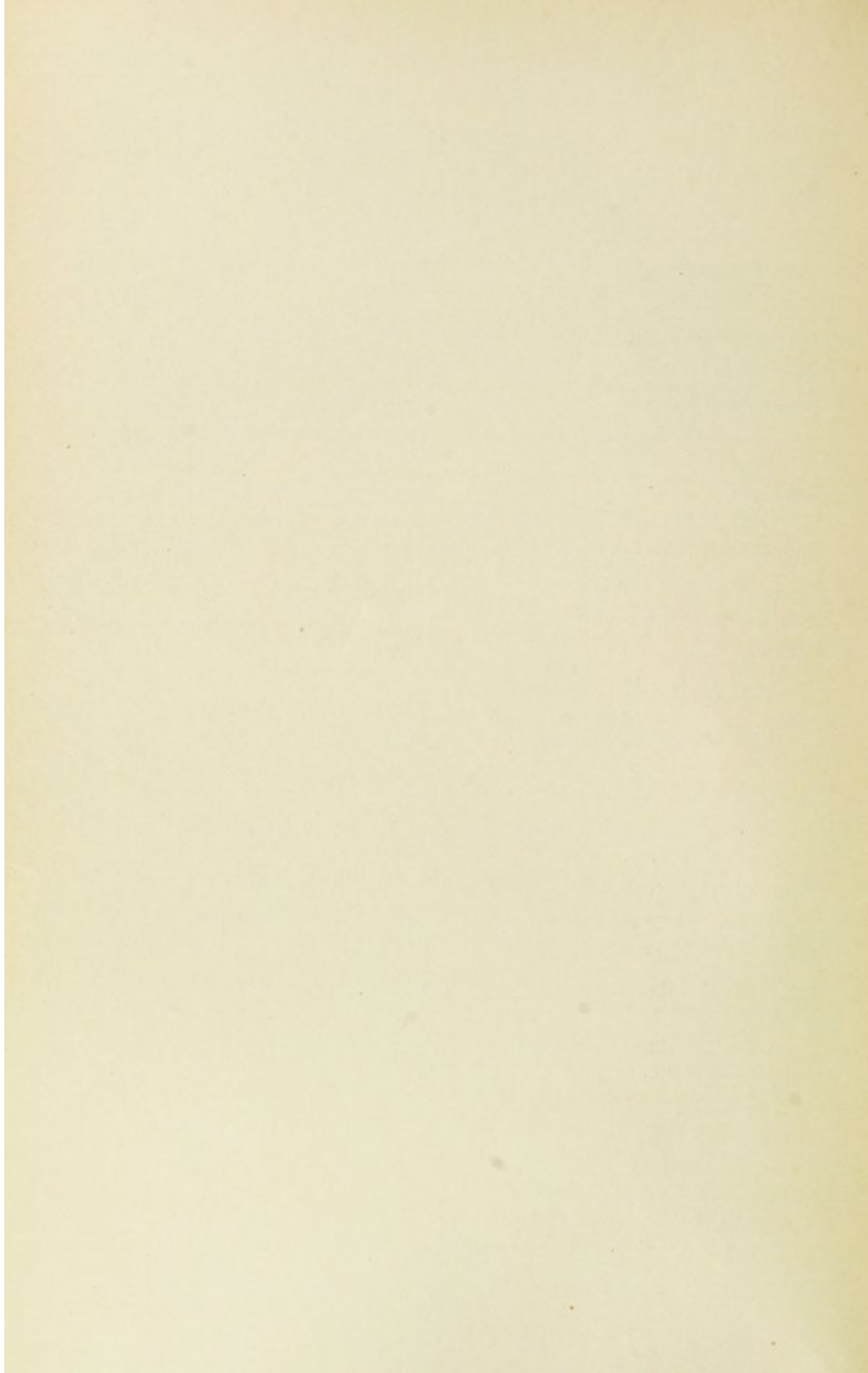
	PAGE
SPECIFIC CHEMOTHERAPY	134-136
Treatment of infections by injections of leucocytic extracts	136
Treatment of infections with normal serum	136-137

CHAPTER V.

SPECIFIC DIAGNOSIS, TREATMENT AND PROPHYLAXIS IN THE DIFFERENT INFECTIONS	138-284
Infections with <i>Mic. pyogenes</i> , streptococci, <i>Mic. pneumoniae</i> , <i>Bact. pneumoniae</i> , the organisms of whooping cough, <i>Bact. influenzae</i> , <i>Dip. intracellularis</i> , <i>Bact. tuberculosis</i> , <i>Bact. Mallei</i> , <i>Bac. typhosus</i> , <i>B. paratyphosus</i> , A and B, <i>Bact. dysenteriae</i> , <i>Bac. coli</i> , <i>Vibrion cholerae</i> , <i>Bac. pestis</i> , <i>Bact. diphtheriae</i> , <i>Bact. titani</i> , the organisms of small-pox and rabies	138-259
Hay fever, autumnal catarrh	259-260
APPENDIX.	
Diagnosis, treatment and prophylaxis in syphilis and malaria	263-284

ILLUSTRATIONS.

FIGURE		PAGE
1.	Graphic representation of receptors and antibodies of the first order .	17
2.	Graphic representation of receptors of the second order .	18
3.	Graphic representation of antibodies of the third order	19
4.	Graphic representation of Ehrlich and Marshall's conception of poly- receptors	21
5.	Schematic drawing to show veins from which blood should be with- drawn for blood cultures, Wassermann test, and so on	38
6.	Needle-pointed glass capsule for puncture and for collection of blood .	42
7.	Method of filling glass capsule	43
8.	Final position of blood in capsule	44
9.	Pipette with pencil mark to indicate one volume	45
10.	Pipette containing four volumes	45
11.	Method of mounting in the hollow-ground slide for microscopic agglutination tests and for determining motility of micro- organisms	48
12.	Precipitin test tube reaction	52
13.	Graphic representation of the theory of the fixation of complement reaction	62
14.	Pipette and watch glass for making uniform suspension of bacteria .	69
15.	Spreader and slide for making smear for opsonic index determination	73
16.	Smear for opsonic index determination	74
17.	Schematic drawing to show how antibody index may be affected by injection of antigen	102
18.	Schematic drawing to show relation of bacteria of the typhoid and colon group	204



CHAPTER I.

INFECTION.

By the term infection we understand the entrance of microparasitic living agents into the body and the manifestation of disease as the result of the multiplication and action of these invading organisms. Any micro-organism that is able to produce symptoms or signs of disease is said to be pathogenic. Infection does not always occur when pathogenic micro-organisms are present in or on the body as is evidenced by the large number of "carriers" of typhoid and diphtheria bacilli. Certain conditions are necessary for the establishment of a bacterial or parasitic disease. These conditions may be broadly divided into two classes: those dependent upon the biological properties of the infecting organisms and those dependent upon the conditions in the host and tissues invaded.

Of the biological characters of importance in the infecting organisms in the production of bacterial disease, the most important are: the ability to multiply in the tissues of the body and the ability to produce poisons or substances harmful either to some or to all of the tissues of the body. The number of organisms necessary to cause disease varies with different species. Many more staphylococci are necessary to cause the formation of a furuncle than anthrax bacilli to cause anthrax. The properties of being able to multiply rapidly and to produce substances poisonous to the body tissues generally determine virulence. That virulence is of great importance is evident from the fact that innumerable so-called saprophytic bacteria, which are present in the different parts of the body, grow and multiply there but, because in their growth sufficient poison is not produced, do not cause an infection. Generally the virulence of pathogenic bacteria decreases on cultivation in artificial media and increases by passage through suitable animals. The virulence of an organism, according to numerous investigators, depends upon the presence of certain substances in the parasite that reduce the resistance of the body and its tissues. These substances are designated by Bail as aggressins.

Besides conditions in the causal organism, certain conditions must exist in the host or body so that infection can occur. Some animals are insusceptible to the action of certain species of micro-organisms; thus, for example, the horse and other domestic animals are naturally immune to venereal diseases. Some organisms can only produce disease in certain parts of the body, while other species require particular portals of entry. The spirillum of cholera, for example, can only produce the typical form of the disease when it has gained entrance through the small intestine; subcutaneous injection with this organism produces no disease. Other bacteria are not so definitely limited to tissues or portals of entry; thus the bacilli producing tuberculosis cause infection when they have entered through the skin, mucous membrane, and so on. *Bacterium diphtheriæ*, *Bact. tuberculosis* and *B. typhosus* show predilections for certain tissues, but will also produce diseased conditions in other parts of the body.

In addition to non-susceptibility the body possesses certain natural barriers to disease that must be overcome before infections can be produced. The unbroken skin usually offers a barrier to infection. Age, sex, race, occupation, and so on at times account for susceptibility to invasion of organisms. The resistance furthermore varies for the different micro-organisms, as is evidenced by the fact that for the tetanus bacillus a wound is necessary, while for the glanders bacillus the slightest abrasion of the mucous membrane will furnish a focus for infection. The body fluids, lymph glands, phagocytes, all offer resistance to infection. These barriers are overcome in various ways and under different conditions, so that while individuals may be immune at one time they may at another time be susceptible to the same infectious agent.

Course of Infections.

From the foregoing it is evident that not only must the infectious organism come in contact with the body tissues to produce disease, but it must penetrate the surface to some extent and must be able to grow, multiply, produce its poison and overcome the resistance of the body. The symptoms and signs of infection do not appear as soon as the pathogenic organisms enter the tissues. The interval between invasion of the tissues

and the appearance of symptoms and signs is known as the incubation period and varies according to the biological characteristics of the infecting organism, but is also influenced by the number and virulence of the organisms, location of the portal of entry and the individual susceptibility of the host. The length of the incubation period varies greatly: in some cholera infections it is as short as a few hours, while the symptoms of rabies may not develop until after six months or a year. In most infections the onset of the disease occurs within one to two weeks. Knowledge of the length of the incubation period is of considerable assistance in diagnosis. At times the actual symptoms and signs of infection are preceded by malaise, headache, and so on, this period being called the prodromal stage.

The course of infection is determined partly by the conditions produced by the specific organism and partly by the distribution of the organisms in the body. It is however to be noted that the course of infection varies for the same micro-organism and that many of these variations we are unable to explain.

After micro-organisms have entered the body their further distribution varies to some extent with the species: thus, staphylococci generally remain localized forming furuncles, carbuncles, and pustules; typhoid bacilli, though causing lesions primarily in the intestines, are present in the circulating blood and other organs of the body during certain stages of the disease; while tetanus bacilli usually remain localized but form a toxin that affects especially the central nervous system. Sometimes there is a local predisposition or a local cause that favors the growth of micro-organisms in a particular part of the body. This spot of lowered or lessened resistance, the *locus minoris resistentiæ*, may offer a vulnerable place without which the micro-organisms gaining access to the body would not survive. That ulcerative endocarditis seldom occurs except where the heart valves have been previously diseased, that subcutaneous fractures become the seat of abscesses and that there may be tuberculous conditions of the hernial sac without tuberculosis in any other part of the body, are well-known facts and are illustrations of the importance of local predisposition.

At times bacteria produce pathological conditions in the lymphatic glands and other organs of the body some distance removed from

the seat of the initial infection: thus micro-organisms may first lodge in one part of the body and produce disease there and from the primary lesion other parts of the body may become infected either through contact of tissues or through transportation by the blood- or lymph-streams. When the blood-stream not only is the carrier of micro-organisms but also becomes the place for growth and reproduction of the same, a septicemia arises. While almost any of these conditions may be produced by any of the pathogenic bacteria, still bacteria show a selective action and distribution. Because of this tendency to produce particular pathological conditions, the prognosis and method of treating infections vary with the species of micro-organism producing the infection.

The infecting micro-organism may produce disease in a mechanical way because of large numbers or because of effects of toxins that may remain localized or be distributed over a large part or the whole of the body. Nearly all pathogenic bacteria cause disease by means of their toxins. Some bacteria, as *Bacterium diphtheriæ* and *Bacterium tetani*, liberate a highly toxic soluble substance in the living body or in the artificial culture medium. This class of poison is known as toxin, extracellular toxin or soluble toxin. Other bacteria, as those causing cholera, typhoid fever and epidemic cerebrospinal meningitis, which are all highly toxic diseases, do not produce toxins that are given off into the culture medium, nor are their toxins supposed to be liberated in the body until the bacterial cells have disintegrated. Such toxins are referred to as intracellular toxins or endotoxins. They are supposed to exert their toxic effects when the bacterial cells are broken up as the result of the bacteriolytic action of the body fluids and white blood cells, especially the phagocytes. It is believed that the conditions for endotoxin formation and liberation are more favorable in the body than in culture media.

At the point of infection or portal of entry the effects produced vary to some extent with the infecting organism, in this way frequently making the diagnosis of the organism tentatively possible. The extent of the reaction at the portal of entry and about the infected area, varies with the prevalence and virulence of the infecting organisms and with the anatomical structure of the part of the body involved. The most commonly seen reaction about infected

areas is that which results in pus formation, in which, due to chemotaxis, leucocytes accumulate. Pus formation and inflammatory reaction as demonstrated in vascularization and proliferation of fixed tissues, are generally regarded as of importance in the protection of the body against invasion by bacteria. At times the local reaction at the portal of entry may be slight or entirely absent as is the case in sleeping sickness and at times in infections with streptococci and anthrax bacilli. In these cases the infections are primarily blood infections, the organisms multiplying in the lymph-glands, in the red blood corpuscles or in the blood plasma. Infection in the bloodstream may be constant until terminated by death or recovery of the patient, as is the case in infection with typhoid bacilli; periodical, as in relapsing fever; or cyclic, as in sleeping sickness and malaria.

Besides the local reaction and that about infected areas, general reactions usually follow in all severe infections. General reactions occur as a result of the action of the toxins produced by the micro-organisms when they are absorbed by body tissues, and although in most instances the bacteria producing the toxin are active in the body tissues and fluids, still it must be recognized that the bacterial toxins may be produced outside the body. This is the case in botulism, the toxin being formed by *B. botulinus* in meat before it is used as food.

The general symptoms produced vary according to the location of the primary lesion, the extent of the process, the peculiarities of the organisms and the resistance of the body tissues. The more common general reactions are fever, leucocytosis, digestive disturbances, effects on the nervous system, anemia and enlargement of the spleen and other glands.

The symptoms and signs produced by an organism will vary as the organism acts locally or generally. In local infections the most marked disturbance occurs at the portal of entry of the micro-organism, while in general infections the reaction manifests itself in the whole or a large part of the body. The appearance of general symptoms varies greatly with the rapidity of production and absorption of the toxin. Experimentally, intravenous and intraperitoneal injections of micro-organisms are followed by symptoms earlier and more consistently than are subcutaneous injections.

Many infections and infectious diseases are self-limited in their

course, recovery taking place after a rather definite period of time has elapsed. When this is not the case the infection becomes chronic and gives rise to varying conditions depending on the species of organism, the part of the body involved, surgical and therapeutic measures resorted to, and so on. The most commonly observed results of chronic infection are ulcers, abscesses, enlarged lymphatic glands, degenerative tissue changes of the various organs, cachexia and slight fever. In some chronic infections there is developed a partial harmony between the parasite and the host, so that the parasite behaves as a saprophyte as far as a particular host is concerned.

The elimination of the causal or etiological factor of infections and infectious diseases occurs in various ways. When the seat of the disease is on the surface of the body the organisms are eliminated with the diseased tissues, but when the organisms are found in parts of the body where this is impossible the micro-organisms or their disintegrated bodies must enter the general circulation and be eliminated with the secretions and excretions. Wyssokowitsch, one of the earliest workers on this subject, believed that bacteria pass into the excretion of an organ only in case the bacteria have done some injury to the organ. He based this assumption partly on the fact that when he found bacteria in the urine there was albuminuria or sometimes hematuria. Sherrington from his experiments concluded that there is always at least a small lesion in the kidney and in nearly all instances albuminuria and hematuria exist in association with the presence of micro-organisms. The kidney is certainly an organ through which bacteria can be excreted. That this can occur without damage to the organ seems to be well established. The liver probably outranks the kidney as an organ for the excretion of bacteria. Welch found that on intravenous injections of colon bacilli into rabbits, the bacilli disappeared from the circulating blood and the organs but made their appearance in the urine and bile. Apparently these bacilli can survive a long time in the bile, but their presence in the gall-bladder usually eventually kills the animal because of the changes in the bile, manifested by its becoming clear, watery, less viscid and by its loss of pigment. In this way infections in the gall-bladder probably form the starting point of chronic concretions. Elimination of bacteria

through the sweat glands has not been definitely proven. Various bacteria are carried by the blood from the intestinal tract and without ever producing a focus for disease are eliminated through the various organs. In the blood and body tissues bacteria are in many cases destroyed by the bacteriolytic and bactericidal substances, although in some instances an immunity or resistance to these may be developed by the bacteria. Sometimes after an apparent recovery from an infection viable organisms remain and later again give rise to disease.

CHAPTER II.

IMMUNITY.

Immunity may be defined as non-susceptibility to a disease or as the ability to resist the action of the causes of the disease. Generally we consider under the term only non-susceptibility to those diseases that we recognize as being due to infection. The body may be immune because of inherited properties or because it has acquired immunity. Immunity because of inherited properties is called "natural" immunity, while the immunity acquired during life is called "acquired" immunity.

Natural immunity is demonstrated by the non-susceptibility of certain animals to the action of some of the micro-organisms causing disease in man. It is an immunity of species, race and at times of family. This immunity in some cases may be reduced or removed by hunger, exposure to cold, exhaustion, etc., and is then only a relative instead of an absolute immunity. By some natural immunity is never regarded as absolute but only relative. Non-susceptibility is frequently called a natural resistance and at times is only an apparent immunity, depending in these cases on the common natural barrier to the entrance and development of disease-producing organisms. Again, what may be regarded as a natural immunity is, in part at least, only a resistance to infection due to the inability of organisms to reach viable tissues. This is the case when the acidity of the stomach is sufficient to kill cholera organisms before they reach the epithelium of the intestine.

Specific acquired immunity results only after a pathological condition exists or has existed. The individual in these cases becomes immune because he has survived a natural course of the disease, as is the case following an attack of scarlet fever; because he has gone through a modified form of the disease, as is the case in vaccination against smallpox; or, because he received substances prepared by some other individual or animal that has gone through a natural or modified course of the disease. Acquired immunity may be life-

long as is the case for smallpox and scarlet fever, or it may be only of short duration as for erysipelas. Two types of acquired immunity are recognized and are referred to as active and passive.

An individual acquires an active immunity to certain micro-organisms when he himself has survived a natural or modified course of the disease produced by infection with this particular micro-organism. The individual in this case produces his own immunity either because he has had the disease naturally or because it has been intentionally and experimentally produced. Experimental, artificial, or intentional active immunization is usually called vaccination and generally produces in the individual mild forms of the symptoms generally found in the infection. Successful intentional immunization with the least discomfort to the individual immunized has been accomplished in various ways, and in practice it has been found that the methods employed must vary for the different organisms. Active, protective and curative immunization is most frequently attempted with injections of killed micro-organisms, although toxins and living organisms are used to immunize against certain infections and infectious diseases. Killed organisms prepared for immunization are frequently referred to as vaccines and bacterins. To prepare them, the micro-organisms are grown on suitable culture media, suspended in salt solution and killed by exposure to heat. The more detailed methods of preparation are referred to in Chapter IV. When living organisms are injected their virulence is usually reduced by passage through suitable animals, growth on certain artificial culture media, incubation at unfavorable temperatures, or in the presence of certain chemicals or under unfavorable conditions as far as oxygen is concerned. Living virulent organisms are at times used for immunization, but in these cases the injections are made in such parts of the body where the disease will not be produced. When killed or living organisms or their toxins are used to confer an intentional active immunity, several injections are usually made and the amounts injected are usually larger in the later than the earlier injections.

Passively acquired immunity is acquired by the introduction of immunizing substances that have been prepared by actively immunized individuals or animals. It is usually conferred by the injection of blood-serum from immunized animals, although it may also be

acquired by the offspring in utero through the placenta or by feeding on milk of an immunized animal. The individual or animal passively acquiring immunity does little or nothing toward obtaining this immunity.

There are several classes of immunizing substances: those acting on bacteria are said to be antibacterial, those acting on toxins are called antitoxic, and those preparing bacteria so phagocytosis can occur are said to be opsonifying or bacteriotropic. Of these classes of immunizing substances antitoxins have been most efficient in passive immunization. Acquired immunity to some infections disappears after a time. The reasons ascribed are many: exhaustion, destruction and excretion of immune substances; production of antibodies by the invading bacteria (Welch); greater ability for absorption of immune bodies by bacteria; overproduction of toxin due to stimulation; and production of cell toxin able to destroy red blood-corpuscles, leucocytes and fixed cells. All these have been given as causes for the disappearance of acquired immunity to some infections.

Theories of Immunity.

Various explanations of the causes and processes of natural and acquired immunity have been attempted. Analyses of the body tissues and fluids have been made and theories formulated for the explanation of the phenomena. Of the many theories only the more important ones are given here.

EXHAUSTION THEORY.—Klebs, Koch and Pasteur tried to explain the changes that occur in the acquisition of immunity by assuming that during immunization certain substances necessary as food for the parasites are used up. If the food necessary for the micro-organisms is consumed the individual acquires an immunity to these organisms. This immunity lasts as long as the food necessary for the parasite's existence and the production of disease is absent from the body.

NOXIOUS RETENTION THEORY.—Chauveau assumed that in immunization bacteria produce substances that are retained in the body of the immunized animal and individual and prevent further multiplication of these organisms. These products protect the body tissues from further invasions by that partic-

ular species of parasite as long as the noxious substances remain.

PHAGOCYtic THEORY OF METCHNIKOFF.—Haeckel in 1858 reported that he had observed that if particles of certain dyes are injected into the veins of molluscs they could soon afterward be found in the blood-cells of these animals. Metchnikoff from various observations concluded that phagocytosis occurs for various purposes, the principal ones being to nourish the cell, to resorb certain cells now useless, to dispose of foreign cells and to protect the cells of the body. The cells capable of engulfing food, cells and foreign bodies he classifies as macrophages and microphages, of which the large mononuclear lymphocytes and the polynuclear leucocytes respectively are the most important in immunity. He further believes that the microphages are of most importance in acute infection, while in tuberculosis the macrophages play the principal part in destroying the causal organisms. Phagocytic activities according to Metchnikoff's views are due to a ferment that he calls cytase, which is thermolabile and under normal conditions exists only in the leucocytes. When phagocytes undergo solution (phagolysis) cytase is liberated and under these conditions is contained in the serum of defibrinated or coagulated blood. All natural immunity according to Metchnikoff is due to phagocytosis and digestion of the micro-organisms, the latter being accomplished by the cytase and fixator both of which are found in the phagocytes and are liberated by them. When immunity to certain bacteria is acquired there is only an increase of phagocytic power, though on immunization there may be a liberation of fixator that may render the bacteria more susceptible to phagocytosis. Antibacterial sera used for passive immunization according to this view are only of value because they stimulate phagocytosis, while antitoxic sera merely stimulate the leucocytes to increased toxin absorption. Metchnikoff's views were first formulated and presented in 1883 and while according to these assumptions, immunity is due to the action of leucocytes that engulf and destroy bacteria and absorb toxin, still his cytase and fixator are probably identical with complement and amboceptor which

he believes are contained in and secreted and excreted by the phagocytes.

CHEMICAL THEORY.—In 1887 Salmon and Smith, Foa and Bonome, Roux and Chamberland, and others found that immunity could be produced, not only by the injection of bacteria but also by the injection of the products of bacterial metabolism. As a result a chemical theory of immunity was advanced according to which the tissues of the body are chemically changed during immunization.

HUMERAL THEORY.—Fodor in 1887 observed that the body fluids, especially the blood of the normal animal or individual, contain certain elements by which they are able to destroy bacteria. Buchner, Behring and Nuttall soon after Fodor's observations also recognized that certain sera are able to destroy some species of bacteria while leaving others unharmed. Nuttall further discovered that heating blood to 60° C., or above, destroyed this germicidal power of fresh normal blood. The substances having the property of destroying certain species of bacteria Buchner called alexins. In 1888 Hericourt and Richet, and in 1889 Babes and Lepp, reported investigations that showed that if the blood-serum from animals that had acquired immunity to certain diseases was injected into other animals, an immunity to these same diseases is conferred. Behring and Kitasato in 1890 reported successful immunization of rats to tetanus by means of injections of blood from rabbits immunized to tetanus. From the work of these investigators there developed the humeral theory of immunity. Brieger and Fränkel discovered that tetanus bacilli, and Roux and Yersin that diphtheria bacilli, when grown on artificial media produce toxins that are soluble and can be separated from the organisms. Behring and Knorr later reported that active immunization of animals follows not only the injections of tetanus bacilli but also results upon proper injections of the toxic substances produced when tetanus organisms are grown on artificial culture medium. Serum from animals immunized by injections of tetanus toxin they found protected against subsequent injections of tetanus bacilli or their products.

The next observation of historical interest is that of Phi-

salix and Bertrand, who in 1891 discovered that if large animals are injected with sublethal doses of cobra venom these animals produce substances that neutralize the venom. This protective substance they called antivenin. The discovery of diphtheria antitoxin took place soon after the discovery of tetanus antitoxin, the observations being made by Behring and Wernicke in 1892.

From the various experiments it was assumed that toxin is neutralized by the immunizing substances in immune serum as acid is neutralized by a base. It was however soon found that there is a marked difference of properties between sera from animals immunized by injections of bacteria not producing a soluble toxin and those immunized to *Bacterium diphtheriæ* and *Bacterium tetanti* or their products on artificial media.

Numerous theories have been advanced to explain the phenomena observed but of all these the side-chain or receptor theory advanced by Ehrlich in 1897 stands out most prominently.

EHRLICH'S SIDE-CHAIN THEORY.—Ehrlich assumes that foods must enter into chemical combination with the cell so as to be assimilated. In order to bring about this combination every living cell must have in addition to its dominating body (Leistungskern), a number of groups of different chemical structure intended for proper combination. The paradigm of this picture is to be found in the benzol ring with its side chains. To these chemical groups Ehrlich gave the name of side-chains or receptors. Their principal function is to convert foreign substances into suitable condition so that they may be assimilated and serve as food for the central or active part of the cell. Receptors however have the greatest variety of function, so that at times they combine the cell with substances that are not foods but actual cell poisons. In any case however very definite relations must exist between the receptor and the substance combined with, the receptors being specific in constitution and thus having a selective action. The combination of receptors with foods is loose and after assimilation of foods the receptors are freed. Anchoring of receptors by toxins results in a loss of receptors and causes in-

jury to the active part of the cell. The injury to the cell varies from impairment of function to death and if enough cells having vital functions to perform are destroyed, death of the organism follows. When however not enough receptors are anchored or the harmful substances are not potent enough to destroy the cell and organisms, immunization generally follows.

In 1896 Weigert advanced the hypothesis that normal physiological function and structure depend on the equilibrium due to the restraint the different cells exert on each other, and that when tissue is lost there is a partial loss of restraint and the body does not only produce enough cells to replace those destroyed but there is an actual overproduction of new tissue. This process is observed in some reptiles where there is an actual overproduction of segments after injury and in the healing of wounds and the formation of scar tissue in man. Ehrlich assumes that when the receptors of cells are injured and thrown out of function the cells produce new receptors and that this regeneration does not stop when all the receptors have been replaced but that an actual excess is produced. This Ehrlich believes leads to a disturbance of the equilibrium of the cells as the result of which the surplus receptors are thrown off. In the lower animals where excessive parts are produced as a result of injury this process can actually be observed. The cast-off receptors retain the power of combining with suitable foreign substances but of course cannot furnish a means of serving for the anchorage of food or deleterious substances to the cell. The free receptors enter the fluids of the body and are known as the antibodies.

The principal function of all receptors and side-chains is to provide for the nutrition and metabolism of the cells. Receptors, and hence immune bodies, however are not all of the same composition or even of the same general structure. In order to explain the different functions and actions of different antibodies, Ehrlich has assumed that the receptors vary in constitution and structure. Any cell of the body may have numbers of the same and different kinds of receptors. Ehrlich divides receptors into three orders. To be able to understand the problems of immunity and the explanations of Ehr-

lich it is necessary to refer again to the manner in which bacteria do harm to the body. Various theories which are now only of historical interest were advanced to explain this and have already been referred to. It soon became evident from the study of the distribution of bacteria in infections that they must injure the body mainly by poisonous substances that are secreted by and liberated from the bacteria. In 1888 Roux and Yersin discovered the first of the bacterial toxins. This they found in the filtrate from broth cultures of the diphtheria bacillus. This toxin they observed was able to produce all the symptoms and lesions of diphtheria except the false membrane. The analogous toxin of the tetanus bacillus was discovered soon after this by Behring and Kitasato. These toxins were different from the ptomaines discovered by Brieger, which were shown not to be specific bacterial toxins. Following the discovery of diphtheria and tetanus toxins it was supposed that other bacteria produce like poisons. We now know that there are relatively few species of bacteria that produce powerful soluble toxins, while a large number produce so-called endotoxins.

True toxins are not ptomaines nor proteid substances. Chemically we know little about them because of their susceptibility to all sorts of injurious agents, so that almost any attempt to precipitate or separate them in any form approaching chemical purity tends to destroy them. While chemical studies have yielded but little, biological researches have been quite fruitful, and now it is fully established that bacterial toxins are very susceptible to heat, acids and alkalies. When dried in vacuo they remain poisonous, but their most distinctive property is the ability to call forth the production of corresponding antibodies known as antitoxins when toxins in suitable quantities are introduced into appropriate animals. In the latter characteristic they differ from the simpler poisons strychnin and morphin. Ehrlich believes that these poisons do not enter into a true chemical combination with the cells. Ford and Abel¹ have shown that the poisonous glucosides of certain toadstools can call forth the production of antitoxin for the glucosid re-

¹Ford and Abel: Jour. Biol. Chem., 1907, Vol. II.

sponsible for at least some of the symptoms of these vegetable poisons. True bacterial toxins are likewise distinguished by their potency and by the characteristic of not manifesting their toxicity until after a period of incubation, in this way again differing from the ordinary alkaloidal poisons.

When we inquire about our knowledge as to the assumed poisons of other infections, such as typhoid fever, cholera, pneumonia and so on, it must be confessed that our information is relatively meager. The organisms of these diseases produce poisons but these apparently do not enter the culture medium on which they are grown, intoxication during the disease being produced by intracellular toxins or endotoxins, which are generally assumed not to be liberated until the bacterial cells die. In spite of this generally accepted theory it is permissible to believe that even though in our cultures on artificial media only endotoxins can be obtained, in the body these bacteria may produce poisonous substances even while they are alive, vigorous and active. The poisons of the bacteria are their weapons of defense and it is reasonable to suppose that in the struggle for existence with the cells and fluids of the living body, these bacteria may be stimulated to produce poisons that they have no occasion to produce on our culture media in which there is no such struggle for existence.

In the studies of toxin-antitoxin combination Ehrlich was able to prove that toxins for which antitoxins could be produced possess two groups, one of which is a combining or haptophore group while the other carries the poison and is called the toxophore group. According to this conception the haptophore group of the toxin is the receptor that combines with a suitable receptor of the cell and in this way brings about a union between the toxin and the cell. It was necessary to assume the presence of the toxophore group because it is possible to destroy the toxic power of a toxin and still not influence the haptophore or combining group. Antitoxin may be bound by toxin molecules which themselves have no toxicity, so that it seems most reasonable to assume that the toxic power resides in a toxophore group. A poison modified by the loss of the toxophore group Ehrlich calls a toxoid, and as

the toxophore group is easily destroyed, toxoids are found frequently in old toxins and toxin subjected to heat or to the action of chemicals. It has also been found that in diphtheria cultures the first toxins produced have a lower degree of toxicity. Inasmuch as the potency of this early toxin is constantly low, these toxins are not really toxoids but have been called toxons, though at first when they were regarded as toxins without the toxophore molecule they were called epitoxoids.

Receptors of the First Order.—Bacterial toxins anchor receptors of this order. Each receptor consists of only the haptophore or combining group that combines directly with the haptophore group of the bacterial toxin. The receptors are of the

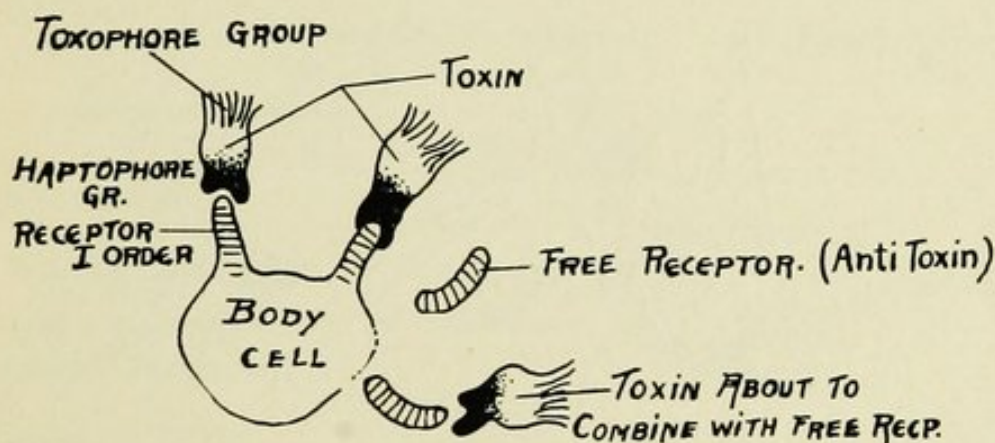


Fig. 1.—Graphic representation of receptors and antibodies of the first order.

simplest character, the haptophore or combining group being the only one of significance to the functions of the cell. During immunization to the soluble bacterial toxins these simple receptors are produced in excess and thrown off. The neutralization of toxin by antitoxin has been principally studied with diphtheria and tetanus toxin and their antitoxins. While Ehrlich has concluded that the reaction is a chemical one, Arrhenius applying the rules of physical chemistry believes that dissociation accompanies combination of toxin and antitoxin, while Bordét believes that absorption accounts for the neutralization of toxin by antitoxin.

This order of receptors and the resulting antibodies are represented graphically in Figure 1. It is on this type of re-

ceptor that the action of the bacterial toxins and the antitoxins of *Bact. diphtheriæ* and *Bact. tetanti* is based.

Receptors of the Second Order.—The receptors of the second order are distinguished from those of the first order in that the receptors here have in addition to the haptophore group, a zymophore, agglutinophore or zymotoxic group. Receptors of this kind, possessing haptophore and zymophore groups, are produced in excess and cast from the cells of the body and circulate in the blood as agglutinins and precipitins during and after immunization to certain bacteria. Production of agglutinating antibodies or agglutinins is supposedly due to stimulation of the cell by agglutinogen which is contained in or secreted by certain bacterial species. Antibodies of this order are not en-

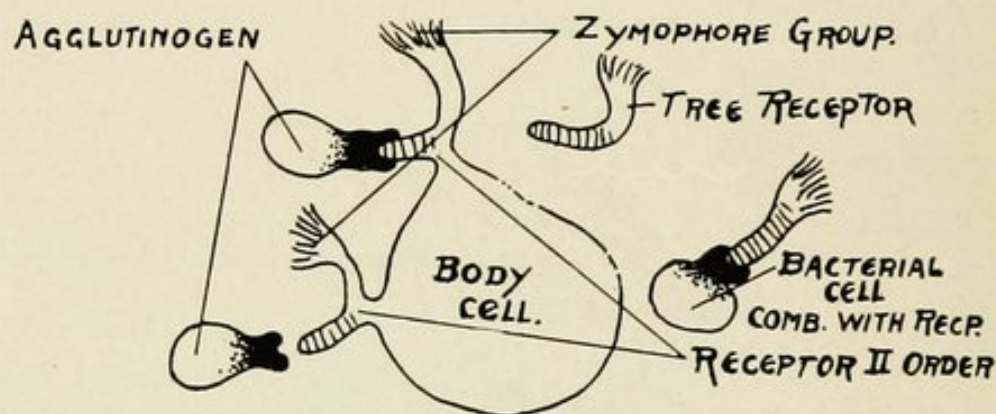


Fig. 2.—Graphic representation of receptors of the second order.

tirely specific for species or variety but rather for groups of organisms closely related in morphology, biology and pathogenicity. This has led to differentiation between common and specific agglutinins and precipitins, of which the specific antibodies are developed earliest and in greatest amounts. In agglutination or precipitation by antibodies the haptophore group combines with the bacterial cells or substance to which the antibodies have been formed, while the zymophore group does not combine with anything but exerts its influence entirely through the attachment made by the haptophore group. In the reaction there are two stages of which the first consists of the union between the haptophore groups of antibodies with substances stimulating the formation of antibodies, while in the

second stage the zymophore groups exert their action. The zymophore group is destroyed by age, acids, heating to 65°C ., and so on. Receptors and antibodies of the second order are represented graphically in Figure 2. They are generally of more importance in diagnosis than in immunization.

Receptors of the Third Order.—The receptors of the third order have two combining groups; one for the anchorage of cells or food substances and the other for combination with a ferment-like substance known as complement or alexin, which is present in normal serum. In immunization receptors having these two combining groups are produced in excess and thrown off by the

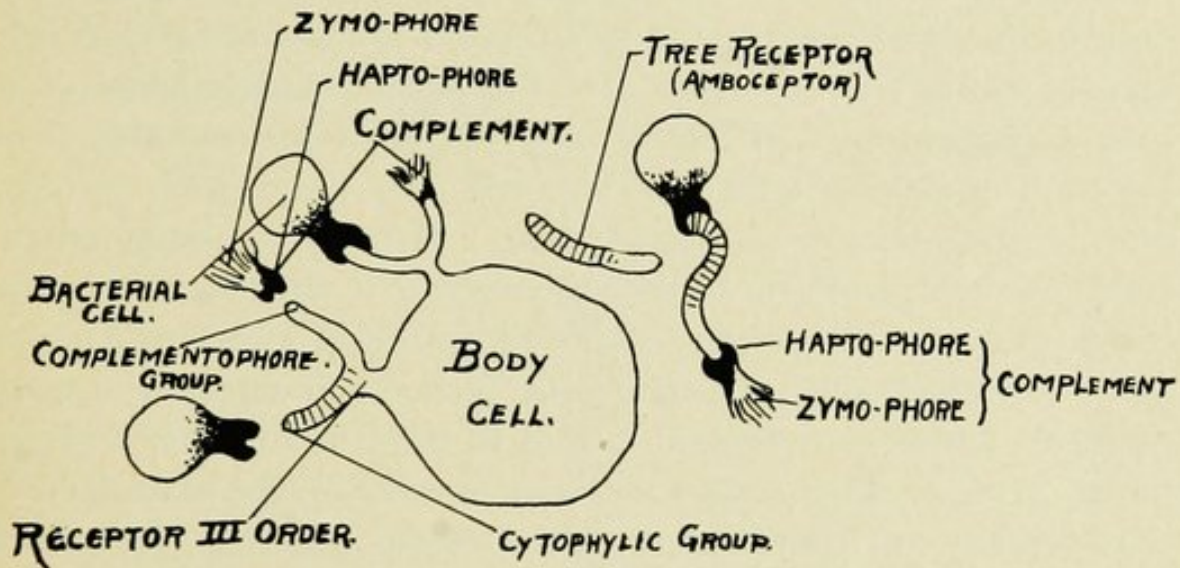


Fig. 3.—Graphic representation of antibodies of the third order.

body cells, while complement, the activating substance, is normally present. The receptors of the third order are graphically represented in Figure 3.

While both amboceptor (the name given to receptors of the third order) and complement are regarded of importance in bactericidal and bacteriolytic sera, still during infection and immunization the amboceptor only is increased. Amboceptor is heat and age resisting while complement, the substance in normal blood that is able to exert its lytic action only through the amboceptor, is easily destroyed by age, acids, heating to 55°C ., and so on. The part taken by antibodies of the third order in immunization is somewhat differently interpreted by

the various investigators. Ehrlich regards amboceptor as introducing a new chemical affinity that attracts complement. Müller's conception of the copula and London's of the desmon are similar to Ehrlich's intermediary body. Bordét on the other hand has regarded the amboceptor as a sensitizing agent, comparing the action of amboceptor and complement to that of a lock with two keys of which one key, the amboceptor, prepares the lock for opening with the second key, the complement. Metchnikoff on the other hand compares the action of amboceptor to a mordant, thus believing it to prepare the antigen for the action of complement. For this reason he calls amboceptors, fixators. Whatever view may be accepted, it is certain that amboceptor renders certain foods and foreign substances (antigen) susceptible to the action of complement, so that amboceptor is generally said to sensitize. As has been stated amboceptor combines on the one hand with antigen and on the other with complement. If however complement combines with one arm of the amboceptor it must have a receptor or haptophore group. This conception of a receptor group alone however will not explain the ferment-like action of complement, hence complement is said to have in addition a zymophore group. This makes our conception of the structure of complement not unlike that of the toxin molecule, for which further proof is furnished by the production of anticomplement when suitable animals are immunized to complement. As has been stated before, the complement action is easily destroyed by heat, acids, age, and so on; but when it is dried without heat its activity can be preserved for a long time. When complement is destroyed or absorbed the serum is said to be inactivated but such serum can again be activated by the addition of fresh normal or immune serum. Because complement is not increased during immunization and is present in normal serum, this normal serum may be used to reactivate old serum containing receptors of the third order. There has been considerable contention as to whether there is a unity or multiplicity of complements. Bordét and Buchner, based on the fact that a normal serum can be used to activate many different varieties of amboceptor, contend that all complement is alike. Ehr-

lich has generally assumed that there is a multiplicity of kinds of complements, basing this assumption on the partial digestion and destruction with heat and so on. According to Ehrlich's view it is not essential to have the proper amboceptor alone but suitable complement must also be present to get the immunity reaction. Ehrlich and Marshall have apparently shown that amboceptor may have more than one complement-

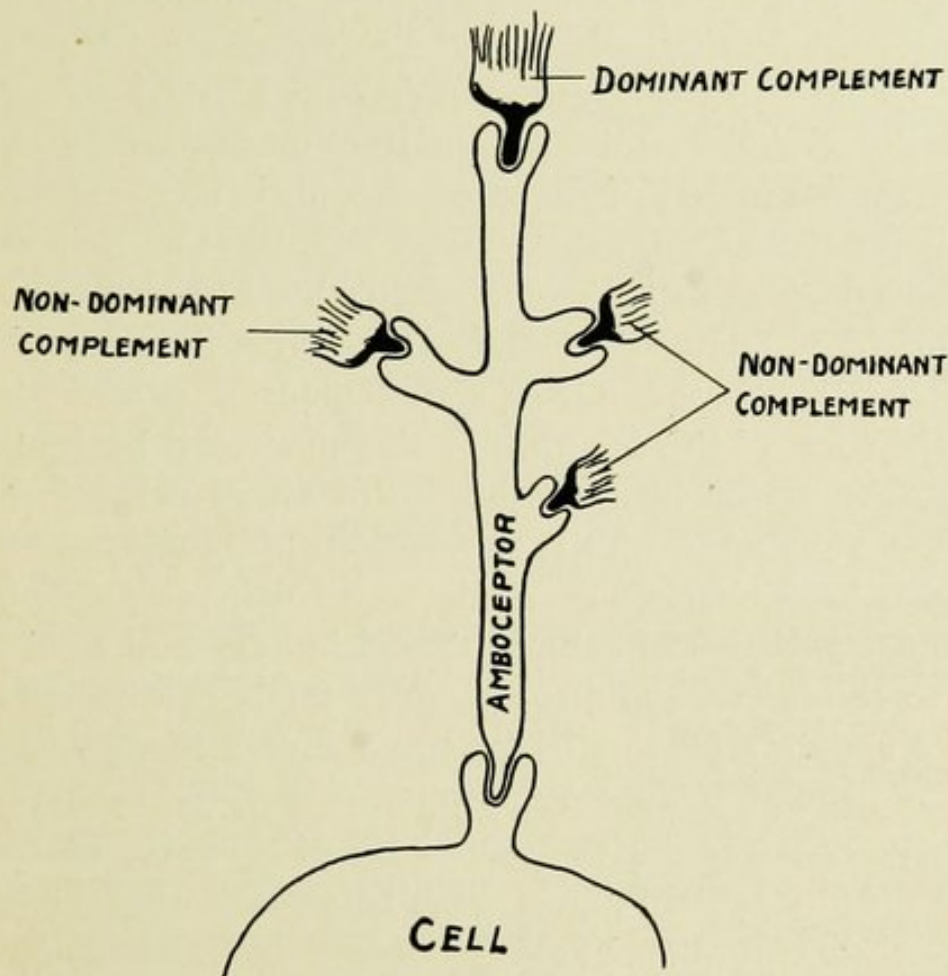


Fig. 4.—Graphic representation of Ehrlich and Marshall's conception of polyceptors.

ophore group; thus there may be a polyceptor instead of an amboceptor. The complement activating serum containing receptors of the third order is called the dominant complement. Ehrlich and Marshall's conception of the polyceptor is shown in Figure 4.

It has further been found that certain salts, as calcium chlorid, may combine with complement and render it inactive but its activity can again be restored by adding suitable chem-

icals. Various investigators, as Hans Sachs, Ternunghi, Hecker and Brand, have shown that complement may be divided into its albumin and globulin portions and that when so divided neither portion has the power to activate. It has further been found that the globulin fraction combines with amboceptor and when combined is thermostable, but when alone or in combination with the albumin fraction it is thermolabile. It is generally thought that the mid-piece of complement is the globulin fraction and the end-piece the albumin fraction. That restoration of complement activity by putting together the globulin and albumin fractions proves that each fraction contains a part of the complement, is not agreed to by Bronfenbrenner and Noguchi¹. These investigators believe that the supposed splitting of complement by hydrochloric acid, carbon dioxid and dialysis is really an inactivation of the whole complement. That the whole complement is present in the albumin fraction of the serum they demonstrated by removing the inhibitory action by the addition of alkali or acid or any amphoteric substance. The conception of receptors of the third order by Ehrlich was made to explain certain physiological characteristics of cells in the assimilation of food. According to this view, while the simpler foods can be taken up readily by simple receptors, the more complex foods with their gigantic molecules cannot be taken up directly by the body cells but the cells require the assistance of certain ferment-like substances in order to digest and assimilate these complex foods. The explanation of the structure and action of amboceptors Ehrlich based on experiments on hemolysis and concluded that the receptors must have two combining or haptophore groups: one for the combination with bacterial or other cells and substances, called the "cytophylic" group; the other for combination with alexin or complement, called the "complementophore" group. All are agreed that it is by means of complement that the amboceptor is able to dissolve bacteria, red blood-cells and other substances. Sera containing free receptors of the third order are bactericidal, bacteriolytic, hemolytic or cytolytic, depending upon whether they together

¹Bronfenbrenner and Noguchi: Jour. Exper. Med., 1912, XV, 625.

with complement kill or dissolve bacteria, red blood-cells or other cells.

It will be observed that receptors of the first, second and third order can be produced in excess to form immune body or antibody. Toxin, bacterial cells, red blood-corpuscles, ferments, complement, and so on, on proper injections into suitable animals lead to the production of antitoxin, antibacterial substance, antiferment, anticomplement, and so on. It has been found that while toxins lose their potency on storage they can still be used in immunization for the production of antitoxin as well as to anchor antitoxin. In a similar manner complement in which the complementophore or zymophore group has been destroyed by heat, age, and so on, referred to as complementoid, can be used to stimulate the formation of anticomplement. As has been stated earlier, immune bodies are formed only for such substances as are able to combine firmly with the receptors of the cells and it is on this assumption that Ehrlich explains the impossibility of producing immunity to certain poisons, as the alkaloids strychnin and morphin.

The cells whose receptors anchor the substances and cells for which immune substances are formed are probably widely distributed throughout the body. The particular tissues in which antibodies are formed have not been definitely determined and probably vary for the different antigens to which immunization can be obtained. Pfeiffer, Marx and Wassermann found that during immunization to cholera and typhoid fever the antibodies occur earliest and in largest amounts in the spleen and blood-forming organs. It is quite definite that in certain cases internal organs may contain more immune substances than does the blood-serum. After immune bodies have been formed they do not remain permanently in the body but are gradually lost through destruction in the body or lost with the excretions.

Toxin and antitoxin produce their effect by simple union, Ehrlich comparing it to a chemical union, but to produce agglutination, precipitation or lysis it is necessary that a ferment-like substance be a part of or be able to combine with the

antibody. This ferment-like substance as stated before is destroyed by heat, age, acids, and so on. It is an integral part of the receptor group of antibodies of the second order, while that for the third order of receptors is present normally in serum. When it has been destroyed or lost the serum is said to be inactive because the receptors of the second and third orders cannot manifest their properties without the help of the ferment-like substances. The complement present in fresh, unheated and untreated normal serum can again activate a serum containing antibodies of the third order but antibodies of the second order cannot be activated by the addition of fresh complement, because the receptors of this order have no combining group for complement. Antibodies are generally specific although some sera contain antibodies for groups of morphologically, biologically and often pathogenically related micro-organisms. In these sera the specific antibodies are produced earliest and most abundantly. The importance of this will be referred to in succeeding chapters. While Ehrlich's theory on the production of antibodies is quite generally accepted, his views concerning the combination and action of toxin-antitoxin, agglutinoids-agglutinins, and of solution and lysis are not so generally agreed to—the whole matter being somewhat unsettled.

It has been demonstrated that antitoxic substances are important in preventing the action of toxins on the body cells, while lytic substances have been found to protect the body by the solution and destruction of bacteria. That the agglutinating or precipitating substances are of importance in destroying bacteria is by no means certain. Numerous investigators assume that agglutinins and precipitins injure and change the bacterial cells, while others claim that they exert no such action. It has been quite definitely observed that it is possible to cultivate and grow bacteria that have undergone agglutination.

OPSONIC OR BACTERIOTROPIC THEORY.—It has been stated earlier that in 1883 Metchnikoff advanced his phagocytic theory of immunity. According to this theory the presence or absence of immunity depends upon the ability of phagocytes to engulf and destroy bacteria. Metchnikoff had given serum little considera-

tion in immunity although he believed that some sera contain substances which stimulate the leucocytes to engulf bacteria. These substances he called "stimulins." The phagocytic theory however was practically replaced for a time by the humeral theory of immunity because Fodor, Buchner, Behring, Nuttall, Ehrlich and others had found that serum containing no cells whatever can destroy bacteria by solution. It was also found that antitoxin is carried in serum.

From 1887 the humeral theory of immunity gained a strong foothold and it was not until 1895 that attention was again called to the function of the leucocytes in immunity. In this year Denys and LeClef reported experiments from which they concluded that in immunization certain changes in the serum make it possible for the leucocytes to engulf bacteria. They based their conclusions on the observations that if normal rabbit serum is added to the leucocytes from a normal rabbit there is only slight phagocytic action for streptococci, while if serum from an animal immunized to streptococci is added to leucocytes from a normal or streptococcus-immune rabbit there is an active engulfing and destruction of the streptococci. Streptococci able to produce erysipelas in a normal rabbit they found would not produce the disease on injection under the skin of a rabbit immunized against streptococci. Protection against streptococci according to these investigators is due especially to the ability of leucocytes to destroy streptococci, the increased phagocytosis being caused by the action of the immune serum on the leucocytes.

The results and conclusions of Denys and LeClef were not agreed to by Bordét. Mennes found that immunity to *Micrococcus pneumoniae* depends upon phagocytosis and that for this serum is necessary; but whether the serum acted on the leucocytes or the bacteria he did not decide.

In 1903 Wright and Douglas pointed out that there are certain substances in sera that so affect bacteria that they are more easily taken up and disposed of by the leucocytes. This substance preparing bacteria so they can be taken up by the leucocytes they called opsonin. Opsonin was found to be present in normal and immune serum and from their investigations, Wright and Douglas decided that the amount of opsonin in serum is variable, is of importance in

infection and can be increased or decreased by injection of killed cultures of bacteria. They express the amount of opsonin in serum in terms of the "opsonic index," which is the ratio of the "phagocytic index" for patient's serum to the phagocytic index for serum from normal individuals. To determine the phagocytic index they modified the method introduced by Leishman in 1902. The methods employed in determining the opsonic index will be taken up in detail in Chapter III.

In 1904 Neufeld and Rimpau, working entirely independently of Wright and Douglas, found that in addition to antitoxic and bactericidal substances there is another factor that is of importance in immunity. Their observations were based on the protective powers of antistreptococcic and antipneumococcic sera in which they found substances that sensitize the corresponding bacteria and so modify them that they are more readily engulfed by the leucocytes. This new substance in immunization they called "bacteriotropin." It is to be noted that they believe bacteriotropin does not act on the leucocytes but exerts a sensitizing influence on the bacteria and furthermore, they regard it as a definite result of immunization. Normal serum they found does not sensitize virulent streptococci or pneumococci so they can be engulfed by the leucocytes as does the serum from properly immunized animals. There is now no doubt that opsonins and bacteriotropins are the same although bacteriotropins are probably what Wright and Douglas have called immune opsonins.

Wright has consistently assumed that normal and immune opsonins are identical and has preferred to regard all opsonins as thermolabile. There are however on the other hand a large number of observations that show that, while opsonins in immune serum resist a temperature of 55° C. for one hour, opsonins in normal serum will no longer be able to prepare bacteria for phagocytosis by the leucocytes after such an exposure to heat. Observations show plainly that immune opsonin is not destroyed by heating to 55° C. for one hour while normal opsonin is. The basis for Wright's assumption that they are all thermolabile is not evident inasmuch as Wright and Reid have shown that in the serum of certain tuberculous patients there is a thermostable opsonin, and on this observation Wright has based a method for the diagnosis of tuberculosis which

can only have for a foundation the assumption that in tuberculosis specific immune bodies that are heat resisting are produced. From the many investigations reported normal and immune opsonins manifest a marked difference in ability to withstand heat.

The existence of normal and immune opsonins is now quite generally accepted. Neufeld in a consideration of the causes of phagocytosis states that he believes bacteria and foreign bodies are only taken up by the leucocytes when there is an appetizer. He bases this assumption on the phagocytosis of red blood-cells by leucocytes. This occurs only when a special hemotropic serum is present in which the physico-chemical condition is so changed that a part of the body is modified to serve as stimulus or appetizer for the phagocytes. Virulent organisms dissolve with greater difficulty and give off less appetizer and because of this there is less phagocytosis of virulent organisms than of organisms with decreased virulence. Spontaneous phagocytosis according to Neufeld is due to changes in the cell which accidentally stimulate the leucocytes to phagocytosis. Normal opsonin is believed by Neufeld to produce its action because of normal amboceptor and complement which gently dissolve bacteria and in this way stimulate the leucocytes to phagocytosis. The assumptions of Neufeld are borne out by numerous other investigators. During immunization Neufeld believes a specific immune substance, bacteriotropin, which modifies bacteria or other cells so that they will serve to stimulate the leucocytes to phagocytosis is produced. Immune opsonin, or bacteriotropin as he prefers to call it, is a thermostable substance that does not require complement.

Normal opsonins, though not acknowledged by Wright, Hektoen and others to be different from immune opsonins, have characteristics by which they differ from immune opsonins. Normal opsonins resemble complement in that they are absorbed or fixed by bacteria, blood-corpuscles, specific precipitates and indifferent bodies and exhibit thermolability and susceptibility to deterioration by age. Noguchi has found that normal opsonins resemble complement in that they are highly labile bodies, lose their action on standing several days, are preserved for a long time when present in blood in a dry state and in this condition can be heated to 135° C. without destruction of their functions. Muir and Martin, Levaditi

and Inman, and Hühne and Neufeld have ascribed the action of normal opsonins to complement. Cowie and Chapin have found that normal guinea-pig serum restores the opsonic power to normal serum that has been heated to 55° C. They believe from their experiments that opsonins (normal?) exert their action because of an amboceptor-complement group. Hektoen has recently published results of experiments from which he concludes that the activating element is free from the opsonin and therefore he believes that opsonins belong to the third order of antibodies of Ehrlich.

Immune opsonins resist temperatures up to 55° C. for one hour, 65° C. at times not being sufficient to destroy their action. If the opsonizing action of immune serum is once lost it cannot be regained by the addition of fresh complement. Muir and Martin have found that inactivated immune opsonin absorbs little or no complement. Because of the properties of immune opsonins they are generally regarded as belonging to the antibodies of the second order of Ehrlich. They apparently possess two groups—the opsonophore and the haptophore. Of these the opsonophore group is destroyed by heat above 55° C., age, acids and so on. It is thus seen that immune opsonins resemble the agglutinins and precipitins in structure and by some investigators have been thought to be identical with agglutinins.

It now seems quite definitely established that the action of normal and immune opsonins is due to entirely different factors in immunity and that immune opsonins are distinct antibodies, probably belonging to those of the second order of Ehrlich. Because the distinction between normal and immune opsonins has not always been made, many of the observations on the opsonic index will have to be repeated before they can be accepted unqualifiedly.

Opsonins have been regarded by numerous investigators, as Savtchenko, Besredka, Loehlein and Dean, as identical with amboceptor (fixateur), but Muir and Martin have shown that not every immune body of the third order produces opsonizing effects and Hektoen from experimental evidence has shown quite conclusively that opsonins are distinct substances or antibodies. Neufeld and Rimpau, Neufeld and Toepfer, Bulloch and Atkin, and others agree with Hektoen, Wright and Douglas that opsonic action is due to the presence of hitherto unknown and distinct substances.

Metchnikoff has described a series of experiments in which the introduction of serum either from normal or immunized animals greatly increased phagocytosis. This action he supposes was exerted on the leucocytes and is of great importance in phagocytic immunity. The substance in the serum that he calls "stimulin" is probably the same as opsonins.

Endotoxins and the aggressins of Bail have by some been supposed to account for the decreased phagocytosis or low opsonic indices obtained with some sera. Aggressins are supposed to injure the leucocytes and inhibit their action. The part the endotoxins play in preventing phagocytosis has not yet been determined. Dorr, Sauerbek and others have shown that aggressins are not definitely specific. Neufeld from experiments has decided that the lack of phagocytosis of virulent organisms does not depend upon the injury to leucocytes and for this reason does not believe that decreased phagocytosis is due to the action of aggressins. It is quite generally accepted that both serum and leucocytes contain substances which, acting after the manner of ferments, are able to dissolve bacteria. Just as all important manifestations of life are found in the normal and pathological cellular elements, so also the means of defense against harmful agents is probably closely related to the condition and functions of cells that prepare and secrete the protective substances by means of which bacteria and other harmful agents are destroyed or neutralized. Hiss, believing that the leucocytes of persons suffering with infections have a lower phagocytic power than those of normal persons, has prepared extracts of leucocytes from normal persons and animals and with these has tried to protect the tissues against invading bacteria. The application and results of this method will be referred to in Chapter IV. Welch a few years ago pointed out that just as the animal body produces antibodies against infecting organisms so also the infecting organisms may produce antibodies which protect them against the cells of the animal body. It is well known that the body often overcomes an infectious disease without entirely ridding itself of the infecting micro-organisms. Deutsch has gone so far as to assume that the increase of virulence of bacteria due to passage through suitable animals is the result of immunization of the bacteria to the animal body. Ehrlich has applied his "atreptic" theory

to explain the resistance of certain organisms to the action of some hosts. According to this theory some organisms do not have proper receptors so they can be affected or do not find the proper receptors in the body cells so they can produce disease. While this conception may at first seem identical with the exhaustion theory of Pasteur still it does not assume that the proper food substances do not exist in the body, but that the bacteria have changed or that the foods exist in such form either normally or after being modified by medication so bacterial cells do not thrive and produce disease. On such assumptions avirulence of micro-organisms, acquired tolerance of micro-organisms to certain therapeutic agents, and changes in bacteria due to passage through certain animals or as a result of cultivation on certain artificial media, may be explained.

CHAPTER III.

SPECIFIC DIAGNOSIS.

Under the term "pathogenic micro-organisms" we include the small living forms that are able to produce disease in man and the animals. They are on the border line of the plant and vegetable kingdoms, belonging especially to the classes of protozoa, bacteria, fungi and yeasts. Of all of these bacteria are the most important and numerous causes of disease.

Loeffler was the first to collect historical facts on bacteriology in 1887. Physicians have always been easily interested in the cause of disease especially at times of pestilences, but most speculations concerning living agent as causes of disease arose in non-medical minds. Lucretius and others said the cause of disease was in living things which were called animalculæ but these ideas were not introduced into medical literature. Fracastorius in the sixteenth century wrote a book in which he almost stated that syphilis is due to some living agent. In the seventeenth century with the introduction of the microscope Malpighi, Swammerdam and others became famous. While Kirchner claimed to have found living organisms in diseased bodies, the communication of Leeuwenhoek in 1683 to the Royal Society is generally regarded as the first description of micro-organisms. Plencig soon after advanced his logical ideas in favor of animate contagion. Müller in the eighteenth century tried to classify and describe the known "bacteria." Pasteur in 1860 finally established the relation of living micro-organisms to certain diseases. The newer methods which Koch advanced in 1876 really mark the beginning of our modern association of certain definite bacterial species with certain diseases. Pasteur again came to the front and using Koch's methods and types of organisms applied himself to various practical problems. He was not interested in morphology or cultural characteristics of bacteria but wanted to find "preven-

tion" and on his investigations are based the modern methods of immunization. He first produced artificial immunity by the use of attenuated organisms in chicken cholera, then in anthrax and finally in rabies or hydrophobia.

With the establishment of the germ theory of disease people at first became prone to regard all bacteria as harmful and to believe that all diseases are caused by bacteria. It is now well realized that bacteria play a most important and necessary part in the activities of nature and that in many of our diseases they play no primary part. Of the many species and varieties of bacteria and other micro-organisms that have been described, relatively few have so far been recognized as the specific causes of particular diseases. By Koch's methods it has become possible to differentiate between bacteria and to learn that bacteria are specific and constant in morphology and characteristics.

As a criterion of the etiological significance of a bacterial species Koch's canons or laws were formulated. Their stipulations are as follows:

1. The species must occur in suitable situations to explain the symptoms and signs in all cases of the disease.
2. Isolation and cultivation of the micro-organisms must be possible.
3. Production of the disease must result upon experimental inoculation of the micro-organism.

From the standpoint of Koch's postulates infectious diseases and their causal agents may be divided into several groups. There is a group of diseases represented by cholera, gonorrhœa, glanders, tuberculosis, anthrax, tetanus, pneumonia and others in which all three conditions of Koch's canons are met. In a second group of infectious but not properly specific diseases belong peritonitis, pleuritis, septicæmia, pyæmia, broncho-pneumonia, pharyngitis, angina, otitis media, and so on. In this group the processes may be due to different causes but usually the etiological factor meets the various conditions of Koch's postulates. A third group of diseases is always accompanied by particular species of micro-organisms which can be cultivated but the disease cannot be reproduced in our laboratory animals. In the fourth group the organisms can be

demonstrated but not cultivated. This group is becoming smaller as methods for artificial cultivation are being improved so that now it is practically only limited to some of the protozoan diseases. By methods that have been devised since Koch formulated his postulates, other lines of proof of the etiological relation of organisms to disease have been established.

There are a considerable number of infectious diseases of unknown etiology. The number of these is gradually being decreased and in regard to the etiology of all of them we have more or less information at present. In regard to some of them—yellow fever, typhus, Texas fever, and so on—we know the animals serving as intermediate hosts and spreaders of the disease. Then there are the problematical bodies found in connection with smallpox, scarlet fever and hydrophobia. It would take a great deal of space to go into details as to what has been done in the study of these bodies but it will be sufficient here to mention that for certain infections they are considered diagnostic. There is a group of diseases as hog cholera, epidemic anterior poliomyelitis, chicken pest and so on caused by what are known as “filterable viruses,” which are usually accepted as meaning that the causal organisms are ultra-microscopic and pass through the pores of the Berkefeld or Chamberland filters. The etiological factors in the group of exanthematous fevers as scarlet fever, measles, chickenpox, smallpox, and so on have presented great difficulties, for not only has it been impossible to find the microorganisms causing them but until recently there has been little success in transmitting them to animals where better and more comprehensive studies can be made.

The medical profession has always been more prone to accept new methods of treatment than methods of diagnosis. To the patient the cure has always been of more importance than the diagnosis. With the increase in the number of diseases in which treatment by particular therapeutic agents having a selective action, specific diagnosis has become of more importance. Unfortunately however much of our specific treatment has been advanced and advocated most ardently by the makers of specific remedies who, realizing that specific

diagnosis of etiological factors is seldom easy, have prepared immune sera and vaccines after the method of "shot gun" prescriptions. The importance of producing specific immune bodies when trying to protect against or recover from infection with a particular species of organisms will be taken up in a subsequent chapter, while here an attempt will be made to consider the various means resorted to in specific diagnosis of infectious diseases.

The best diagnosis for infectious disease is based on the identification of the recognized etiological factor. In some cases the micro-organism can be demonstrated and identified at once but usually twelve to forty-eight hours must elapse before it can be positively identified. As this period of time necessary to make the diagnosis may be of the utmost importance to the patient or the rest of the community, a tentative diagnosis of the etiology of the disease should be made from the symptoms and signs. Again the diagnosis may have to be based on the presence or absence of specific antibodies, reaction of the patient or some part of the body to certain biological, chemical or empirical tests. In some cases the prevalence of a communicable disease, together with symptoms and signs of disease in the patient, may offer the first and only means of diagnosis.

Probably in no field of medicine does the practitioner realize his incompetency to do the required work as in the clinical laboratory. Very frequently the methods are such that the man without special laboratory training cannot comprehend nor execute them. Some clinicians are willing to acknowledge the benefits that may be obtained from laboratory examinations and investigations but are not willing to study them sufficiently to comprehend the interpretations and deductions from the results of the same; while others look upon them as fads entirely unnecessary to the practice of medicine. To obtain the best assistance from the laboratory the clinician should possess enough information on the subject to properly select the laboratory man that is to make the investigation, to properly select the material to send for examination and to interpret the reports he received from the laboratory.

The laboratory man very frequently is asked by the clinician not only for his diagnosis but also for his recommendation of treatment. Inability of the physician to make these examinations for himself and to interpret the results as given to him by the laboratory man undoubtedly at times leads to an injustice to the patient because the diagnosis is held up for some time as well as subjects him to unnecessary expense. In some instances the clinician is unjust enough to the laboratory man to expect him to make these examinations without charge while the physician charges regular fees for his services and at times even for examinations which he himself does not do or expect to pay for. The dangers arising from the clinician's failure to do such laboratory examinations as are accessible to him cannot be over emphasized and whether it be due to lack of knowledge or to the lack of time, the clinician ought to so train and prepare himself that he can make and interpret the results of these examinations. To best accomplish this, it is necessary that the clinician have accurate methods which can be performed in the shortest time possible, as well as train himself and become acquainted with the epidemiology, pathology, and symptoms and signs occurring in infections with special micro-organisms.

I. Isolation and Identification of Etiological Factors.

Of all methods of diagnosis this is the most satisfactory and reliable. The amount of work and apparatus necessary to make a diagnosis possible varies for the different organisms. Various means are adopted for identifying micro-organisms and are of varying importance in different cases.

1. DIRECT MICROSCOPIC EXAMINATION OF MATERIAL.—While usually stained slide preparations are examined, in some cases unstained and unfixed specimens are preferred. This is especially true in searching for *amœba coli* and other intestinal parasites and the parasites of malaria. When stained specimens are desired the material must be spread thinly, fixed with methyl alcohol or one of the stains containing methyl alcohol (Leishman's, Wright's or Hasting's stains), or else they must be fixed by heat. After this properly selected stains must be

used. A positive diagnosis of the causal organism is especially valuable in properly stained slides for the diagnosis of tuberculosis, malaria and sleeping sickness, while for diphtheria, streptococcus, *Mic. gonorrhæa* and staphylococcus infections only a good tentative diagnosis can be made. For most of the other micro-organisms the morphological and staining characteristics are merely aids to the diagnosis.

2. CULTURAL METHODS AND CHARACTERISTICS.—Before cultural characteristics can be obtained it is absolutely necessary that pure cultures be obtained. This cannot be over-emphasized. In making such separations however it must be remembered that the real etiological factor may constitute only a relatively small portion of the total bacterial flora present so that by the process of dilution it may be lost. Again the causal organism may grow only slowly or not at all on artificial media. To overcome these difficulties so-called enriching fluids may be used to increase the proportions of these organisms, as is the case when attempts are made to get *Bac. coli* from water; or animals may be inoculated to get the causal organism in pure culture as is the case in the use of rabbits for the isolation of the gas bacillus and *Mic. pneumoniae*. For micro-organisms growing only slowly longer incubation periods must be allowed. The technique of isolation not only varies for the different micro-organisms but is also dependent upon the material from which the species are to be isolated. Even after the various species have been separated, the details of which are given in text-books on bacteriology and some of which will be described in a later chapter, there is some difficulty at times in determining which of the organisms is the causal factor. After pure cultures of the organisms have been obtained on artificial media, identification is made from morphological, staining, cultural and at times biological characteristics. To determine these characteristics standard methods must be used and correct observations must be made. It is not nearly as important to remember the characteristics of any organisms as it is to properly interpret the results obtained. In recent years fermentation of the different carbo-hydrates, which tests are most conveniently made in Hiss' serum water medium, have

been of great importance in the diagnosis of bacterial species and variety. In all cases it must be remembered that the important species of micro-organisms may not grow on ordinary media. For this reason some material should always be preserved on ice so that cultures can be made on blood and blood serum media or can be grown anaerobically and aerobically as may be necessary. For details in regard to this technique reference should be made preferably to laboratory guides.

Blood cultures for diagnosis have within more recent times become of great importance because in the very earliest stages of infection with some organisms, notably *Bac. typhosus*, a diagnosis can be made some days before a serum diagnosis is possible. For other infections in which serum diagnosis is of little help as for the pyogenic organisms, blood cultures at times are of the greatest help. Because of this importance the general technique of the procedure is given here. It is to be understood that the observer must have knowledge of the general principles of bacteriology and be able to modify the technique here given to suit the particular species in question. Before obtaining the blood it is necessary to have the media prepared and put up in suitable tubes, flasks and plates. Tubes of molten agar cooled to about 43° C. to 45° C. are used for making plates. Bouillon or litmus milk should be put up in 100 c. c. flasks. When typhoid bacilli are to be isolated, ox bile peptone medium is preferred by some. When the blood has been obtained the first agar tubes should be inoculated with about 0.5 c. c. of blood and from these greater dilutions should be made, after which all of the plates should be immediately poured. The flasks should be inoculated with from 1.0 c. c. to 5.0 c. c. of blood. The amount of blood used is determined by two factors: the amount of antibody and bactericidal substance present in the serum and the extent of blood infection. For these reasons some dilutions of serum should at least be greater than 1:100 while others should contain the maximum of serum that can possibly be used.

To obtain the blood a syringe with a capacity of at least 10 c. c. but preferably 20 c. c. should be used. All-glass

syringes are best for this purpose. The regular technique of aseptic operations should be used. Blood is usually best obtained from the median basilic or cephalic vein, the hypodermic needle being passed through the skin into the vein. This can be done easily if a tourniquet is put above the elbow to cause the veins to stand out prominently so they can be held steadily between the fingers while the needle is being inserted. Inasmuch as the veins may be more prominent on one arm than

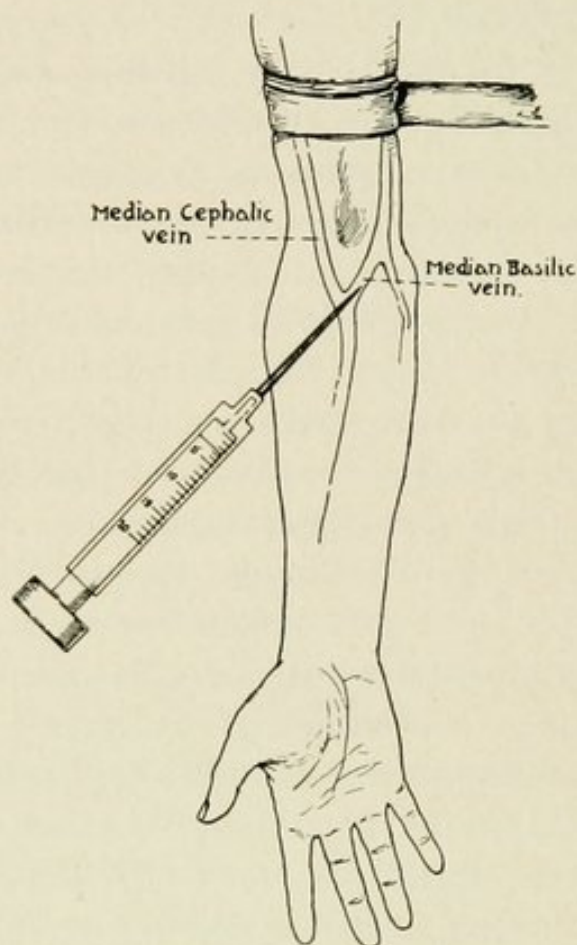


Fig. 5.—Schematic drawing to show veins from which blood should be withdrawn for blood cultures, Wassermann test, and so on.

on the other, it is best to examine the patient before cleaning up or better still to clean up both arms. After the blood has been withdrawn the tourniquet should be relieved, the needle withdrawn, the puncture sealed with collodion and the culture medium be immediately inoculated before clotting of the blood occurs. Wollstein¹ takes blood from the external jugular vein in making cultures from the blood of infants, the entrance

¹Wollstein: Am. Jour. of Dis. of Child., 1912, IV, p. 197.

into the veins being most readily effected during a paroxysm of crying.

Plates and flasks should be examined daily for several days. Usually only one species of micro-organism is found in blood cultures for in the blood stream mixed infection is rare.

3. SERUM REACTIONS FOR THE IDENTIFICATION OF BACTERIA.—For a number of different species of micro-organisms, especially the bacilli of typhoid fever, dysentery, Malta fever, cholera, and so on, it has been shown that for identification, agglutination tests with a specific immune serum are of great importance. Precipitin, bacteriolysin, fixation of complement and other tests depending upon the presence of specific antibodies are also resorted to for purposes of identification. In all cases it must be remembered that specific and common antibodies are formed during immunization so that absorption of common antibodies may be necessary and that various dilutions of antisera are usually used in these tests. To make these tests various dilutions of blood serum from animals containing specific antibodies as a result of immunization are mixed with the bacteria to be identified. Agglutination, precipitation, lysis and fixation of complement will only occur if the bacteria in question correspond to or will anchor the antibodies in the known antiserum. The technique will be described more in detail under serum diagnosis of diseases.

4. ANIMAL INOCULATIONS.—They are resorted to for various purposes. (1) To aid in getting pure cultures as for the gas bacillus and *Mic. pneumoniae*. (2) To furnish a suitable medium for the growth of certain species as for *Bact. tuberculosis*. (3) To serve as an enriching medium as for *Bact. tuberculosis*. (4) To test pathogenicity as for streptococci, tubercle bacilli and so on. (5) To furnish antiserum as a result of immunization.

II. Determination of Etiological Factor by the Presence or Absence of Specific Antibodies.

The body protects itself against invasion by micro-organisms by means of antibodies. In a previous chapter the theories of production of antibodies have been considered and immunity has been divided into antitoxic and antibacterial. These anti-

bodies are formed during infection and are found principally in the blood serum during and after infection. Attempts have been made to introduce these prepared bodies into the infected individual, such treatment being known as serum therapy; likewise attempts have been made to diagnose specifically the organisms causing infection by determination of the presence or absence of their appropriate antibodies, such methods of diagnosis being known as serum diagnosis. It was stated earlier that antibodies are usually specific for micro-organisms, or at least for groups of micro-organisms, and that for the same micro-organisms antibodies belonging to the groups of anti-toxins, agglutinins, precipitins, opsonins, lysins and so on may be formed. Some micro-organisms call forth the production of only one type of antibody while others call forth the production of various types of antibodies. Usually however for serum diagnosis the determination of the presence or absence of some one of the types of antibodies has been found most satisfactory. In all cases it must be remembered that antibodies are not present in blood serum in appreciable amounts as soon as infection occurs. The different antibodies do not appear at the same time, thus Wright and his pupils have found that the opsonic index begins to rise two to five days after experimental inoculation and usually reaches its height after about five to eight days. The appearance of appreciable amounts of other antibodies comes somewhat later. Bacteri-olysins are found to be present at times in the spleen within four hours after experimental inoculation but do not appear in quantity in the blood serum until after five to nine or fourteen days. Agglutinins are present in the blood serum from eight to twelve days after experimental injections of cultures, while typhoid agglutinins usually do not appear in a typhoid patient's blood until after ten days of the disease have elapsed. In practically all cases serum diagnosis alone is not sufficient and the results must usually only be given the importance of one of the cardinal points in the diagnosis.

The principal tests for the determination of specific antibodies in serum for diagnostic purposes are those for the presence or absence of agglutinins, precipitins, opsonins, lysins

and the fixation of complement. The technique for these various determinations varies to some extent.

Collection and Preservation of Serum to be Tested.—Serum for diagnostic purposes may be obtained and preserved in various ways. It is always to be remembered that heat, age and certain chemicals destroy complement; furthermore heating above 65° C. destroys the zymophore portion of the antibodies of the second order. When fresh serum is dried without heating, complement may be preserved for a long time. All serum must be so collected and preserved that accurate dilutions can be made. Inasmuch as the serum is usually diluted further with the culture suspension in making the serum dilutions, this must be taken into consideration. Thus if agglutination of *Bac. typhosus* is to be tested for microscopically in serum dilutions of 1:10 and 1:50, the serum is diluted 1:5 and 1:25; then one loop-full of the diluted serum is added to one loop-full of typhoid bacillus suspension thus making dilutions 1:10 and 1:50.

Dried Blood.—For purposes of convenience in sending blood serum to the laboratory and to prevent deterioration, drops of blood obtained from a puncture in the lobe of the ear or from the toe or finger are collected on strips of mica, aluminum, tin, cover glasses or glass slides and allowed to dry. For making dilutions various methods are used, those based on weight are the most accurate. A more convenient method which gives fairly reliable dilutions is to make a platinum loop the size of the dried drop of blood and then to dilute with the desired number of loop-fulls of isotonic salt solution (0.8 per cent). These dilutions must be allowed to stand so the red blood corpuscles can settle out.

Blood Serum.—Fluid blood serum is obtained in various ways. One is to puncture the median basilic or cephalic vein as for a blood culture (see p. 38) and then emptying the blood obtained into a sterile test tube, allowing it to clot, or else centrifuging it to throw down the corpuscles. Another though less impressive method as far as the patient is concerned is as follows: a small glass capsule with one curved capillary limb is made as is indicated in Fig. 6. This tube is brought to a needle's point at "b" by drawing it out after melting over the

pilot flame of a Bunsen burner or even in the flame of a match. The point is later used to puncture the finger or lobe of the ear from which blood is to be obtained. The end "a" of the capsule is left open. Preferably blood is obtained from the middle finger. After cleaning the finger with alcohol and water a bandage, handkerchief or rubber band is firmly wound round, the windings starting at the base of the finger and running gradually toward the tip as the windings are put on. In this way there is produced an accumulation of blood in the tip of the finger which is now pricked with the needle point of the capillary bulb "b." As soon as the blood starts to flow from the puncture the needle end of the bulb is broken off and the open end "a" is held close to the drop of blood, in this way gradually

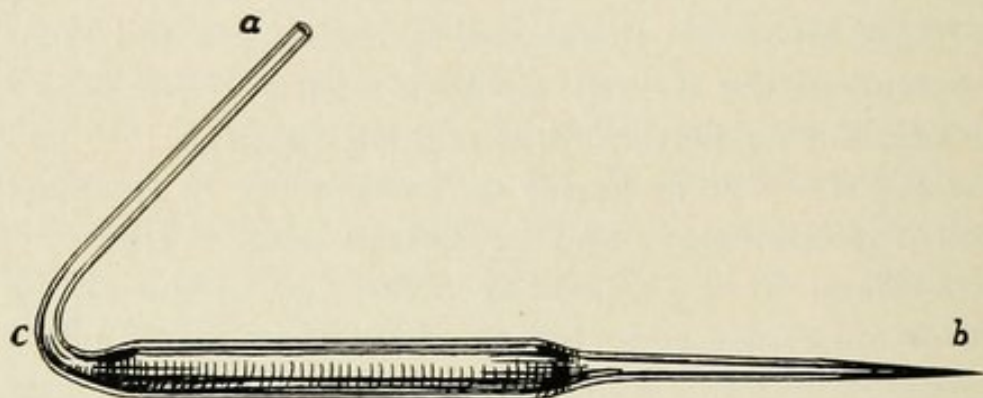


Fig. 6.—Needle-pointed glass capsule for puncture and for collection of blood.

filling the bulb as is indicated in Fig. 7. After a sufficient amount of blood has been drawn and collected in the bulb the end "b" is heated and sealed off in the pilot flame. In doing this the bulb is held between the fingers at "a" and "c" so as to avoid heating the blood in the tube. As the end "b" cools off the blood will be drawn away from the end "a." When the end "b" is cold and all of the blood is in the bulb, the tube is given a rapid swing of the arm as is practiced in shaking down a clinical thermometer and in this way the blood is thrown down into the sealed end "b" as is indicated in Fig. 8. The blood is now allowed to clot in order to express the serum. If haste is necessary the serum may be separated by hanging the bulb at "c" over the arm of a centrifuge and centrifuging

until the clear serum has been obtained. To gain access to the serum the tube is opened by filing and breaking at "c."

Blood may be drawn from the vessels in the lobe of the ear though it usually is more difficult to get sufficient amounts in this way. The curved tube of Wright's is much preferred over the straight tube with a capillary end.

Various methods are in use for making dilutions of the serum obtained. Graduated pipettes, the mixing pipette as used for counting white and red cells, syringes and cylinders are em-

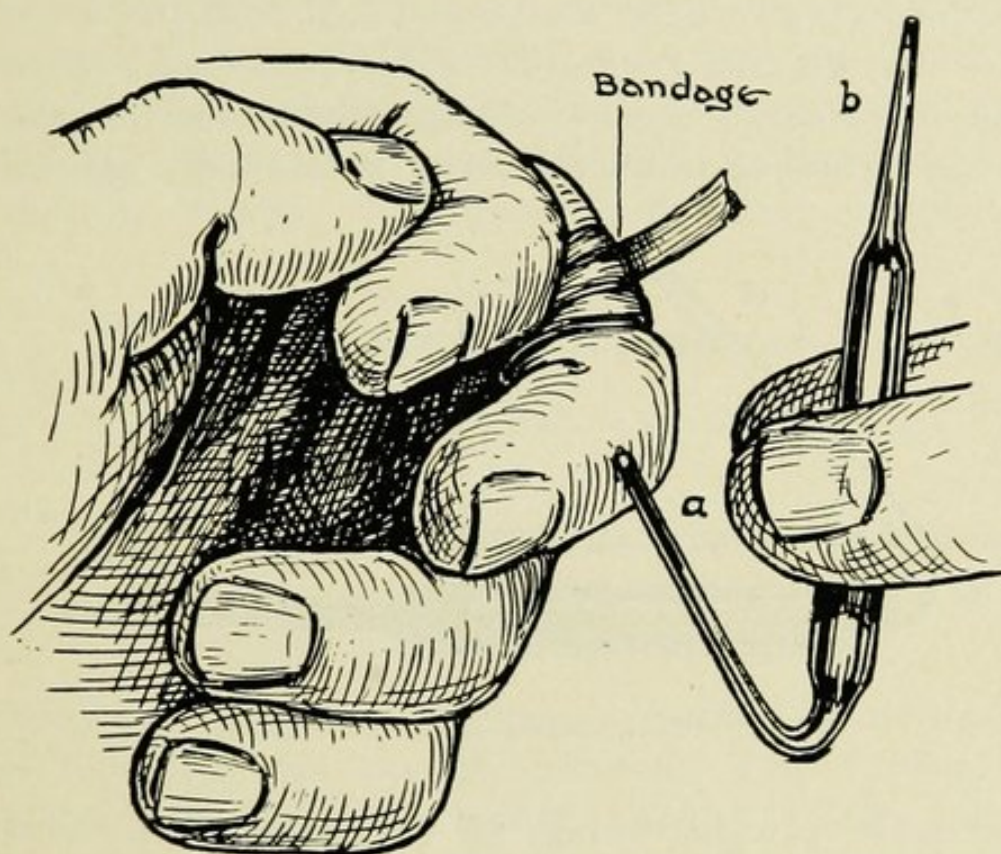


Fig. 7.—Method of filling glass capsule.

ployed for this purpose. Wright and Douglas' method is especially convenient when only small amounts of serum are available. A capillary pipette the walls of which are made thick by thoroughly melting the glass before drawing it out is used. The pipette is broken off squarely after which a mark with a soft wax pencil is made on the capillary end from a quarter to one-half inch from this end. A pipette of this kind is shown in Fig. 9. By means of a rubber teat which is attached to the large end of the pipette, serum is drawn in

until it reaches the pencil mark; the pipette is now withdrawn and the pressure on the teat is released sufficiently to draw the serum up into the capillary about one-quarter of an inch. Then the small end is immersed in the diluting fluid (isotonic salt solution) which is drawn up into the tube as far as the pencil mark. Again the tube is withdrawn and a small amount of air drawn into the capillary tube. As many volumes of diluting fluid are drawn in this way as is necessary to get the dilution of serum desired, after which serum and diluent are forced out of the pipette into a watch glass and mixed well with the aid of the pipette. In Fig. 10 a dilution of 1:4 is shown. A certain amount of practice is needed to make dilutions in this way but when the technique is once learned it is a very satisfactory

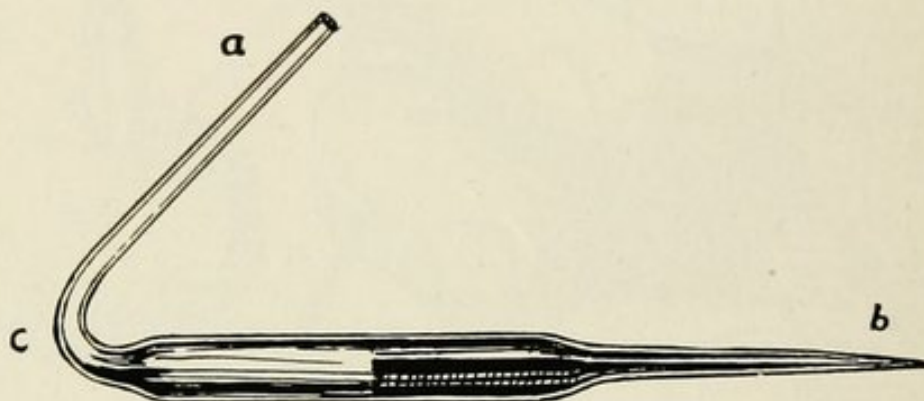


Fig. 8.—Final position of blood in capsule.

method. The principal things to remember are to compress the bulb only partly and to hold the pipette firmly between the third and fourth fingers so that the pipette does not sway at the bulb, the only change in pressure being that exerted by the thumb and second finger on the bulb.

Frequently greater dilutions of serum are made by further diluting low dilutions. This may be done by any of the methods mentioned. In all cases thorough mixing of serum and diluent are essential.

Agglutination Test.—Agglutination occurs because of the presence of so-called agglutinins which belong to the antibodies of the second order as described on page 18. They are receptors having a single combining group to which something in

the nature of an enzyme is attached. The agglutinins have a very wide field of application in the diagnosis of bacterial diseases, the reaction having a two-fold importance being used both in the diagnosis of specific antibodies and the diagnosis of micro-organisms. Since the diagnosis of micro-organisms has already been considered on page 39, only the diagnosis of disease by the reaction is considered here.

The discovery of the phenomenon of agglutination was made by Gruber and Durham who were studying the so-called Pfeiffer reaction as it relates to immunity in Asiatic cholera. They found as early as 1894 that serum from an animal vaccinated against cholera when added to a suspension of cholera spirilla will cause them to cease their motility and to run together in clumps. This phenomenon they designated as

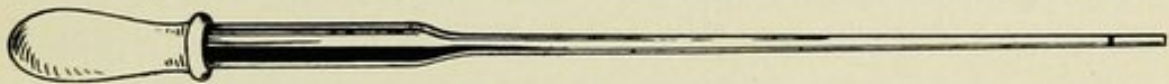


Fig. 9.—Pipette with pencil mark to indicate one volume.



Fig. 10.—Pipette containing four volumes.

agglutination. These investigators further found that the serum from patients having recovered from typhoid fever causes the agglutination of typhoid bacilli. Widal, soon after Gruber and Durham's discoveries which were confirmed by Pfeiffer, demonstrated that the agglutinating property of serum persists not only after typhoid fever has subsided but that it is present during the course of the disease. On this observation is based the so-called Widal reaction for the diagnosis of typhoid fever. For various reasons it is to be regretted that this test should be known as the Widal test because the agglutination test for the typhoid fever diagnosis is only one application of the agglutination phenomenon. Widal was not the first to observe agglutination and he was not even the first to realize that the reaction had a diagnostic value in typhoid fever. Grünbaum had recognized its value before Widal but

was less fortunate than Widal because his work was published some time after that of Widal. It is probably best to refer to the test as "the agglutination test for typhoid fever, cholera, dysentery and so on" as the case may be.

Agglutination tests are either microscopic or macroscopic. For purposes of diagnosis of disease the microscopic method is preferred because the reaction occurs earlier, requires less material and affords the observer better opportunities to detect reactions which even though only suggestive at least warrant further and more careful observations. The macroscopic test however is preferred when the diagnosis of microorganisms is to be made with the aid of specific antisera. The technique and interpretation of these tests varies in the different laboratories.

BACTERIAL SUSPENSIONS.—To make the tests the best results are obtained if the cultures have been recently grown (for typhoid bacillus agglutination 12 to 24 hours of incubation is best) on slanted agar or one of the solid media. The bacteria are then suspended in physiological salt solution or bouillon by pouring a small amount of either of these fluids over the culture and breaking them loose with a sterile platinum needle. Making of suspensions of the bacteria may be overcome by growing the culture in bouillon. The density of the bacterial suspension varies somewhat for microscopic and macroscopic tests. In all cases there should be enough bacteria in the suspension to make the observation clear-cut and still there should not be more bacteria than the serum can furnish agglutinins for. For diagnostic purposes in practice suspensions for microscopical examination should be barely cloudy while for macroscopical examination the suspension should be quite opaque (same opacity as observed in a suspension of 0.3 grams of barium sulphate to 100 c. c. distilled water).

While at first agglutination tests were made with fresh cultures, Widal¹ as early as 1897 observed that the phenomenon occurs equally well with dead cultures. Cultures killed with formalin are used quite extensively for the agglutination test for the diagnosis of typhoid fever. The suspensions are made

¹Widal: *Compt. rend. Soc. de biol., Par.* 1897.

in the regular way and 0.1 per cent. of formalin is added. The method has various advantages among which the most important are that sufficient suspensions can be made at one time to furnish a uniform suspension for many tests, that the worker can accustom himself to the suspension, that the physician can use the test readily in his office, that there is no danger of infection with the suspension and that in this way the necessity of constantly keeping fresh cultures in stock is obviated.

Inasmuch as the clumping of bacteria indicates a positive reaction, it is very essential that control preparations be made. These are made just as the regular preparations except that instead of the various dilutions of serum to be tested similar amounts of isotonic salt solution are used. Whenever there is clumping without the addition of serum the bacterial suspension is at fault. To remedy this cultures on solid medium, further grinding and breaking up of clumps and centrifugalization sufficient to throw down the clumps are resorted to.

(a) *Microscopic Agglutination Test*.—This is usually made by mixing one loop of the bacterial suspension with one loop of the diluted serum. Then the edge of the well in a hollow-ground slide is lubricated with a small amount of vaseline or olive oil, the slide is inverted and so placed over the mixture of bacterial suspension and serum on the cover glass that the drop comes in the middle of the well. Then the slide is again turned right side up. After the slide has been turned right side up the drop should hang from the cover glass and the edge of the cover should be entirely sealed, to accomplish which it may be necessary to add a drop of olive oil at some part of the cover glass. The preparations for the various serum dilutions and controls are made after the same manner.

(b) *Macroscopic Agglutination Test*.—For macroscopic agglutination tests mixtures of suspensions of the bacteria and diluted serum are made. To make the mixtures small pipettes or droppers are used instead of the platinum loop. These mixtures are then stored and observed from time to time. Generally small test tubes are used. They may be of any size but those measuring 0.75 by 10.0 c. m. are the most satisfactory. While

there is no rule in regard to the size of test tube to be used it is always best to use the same size of tube for all tests for the series of dilutions of serum as well as for the control tests. In

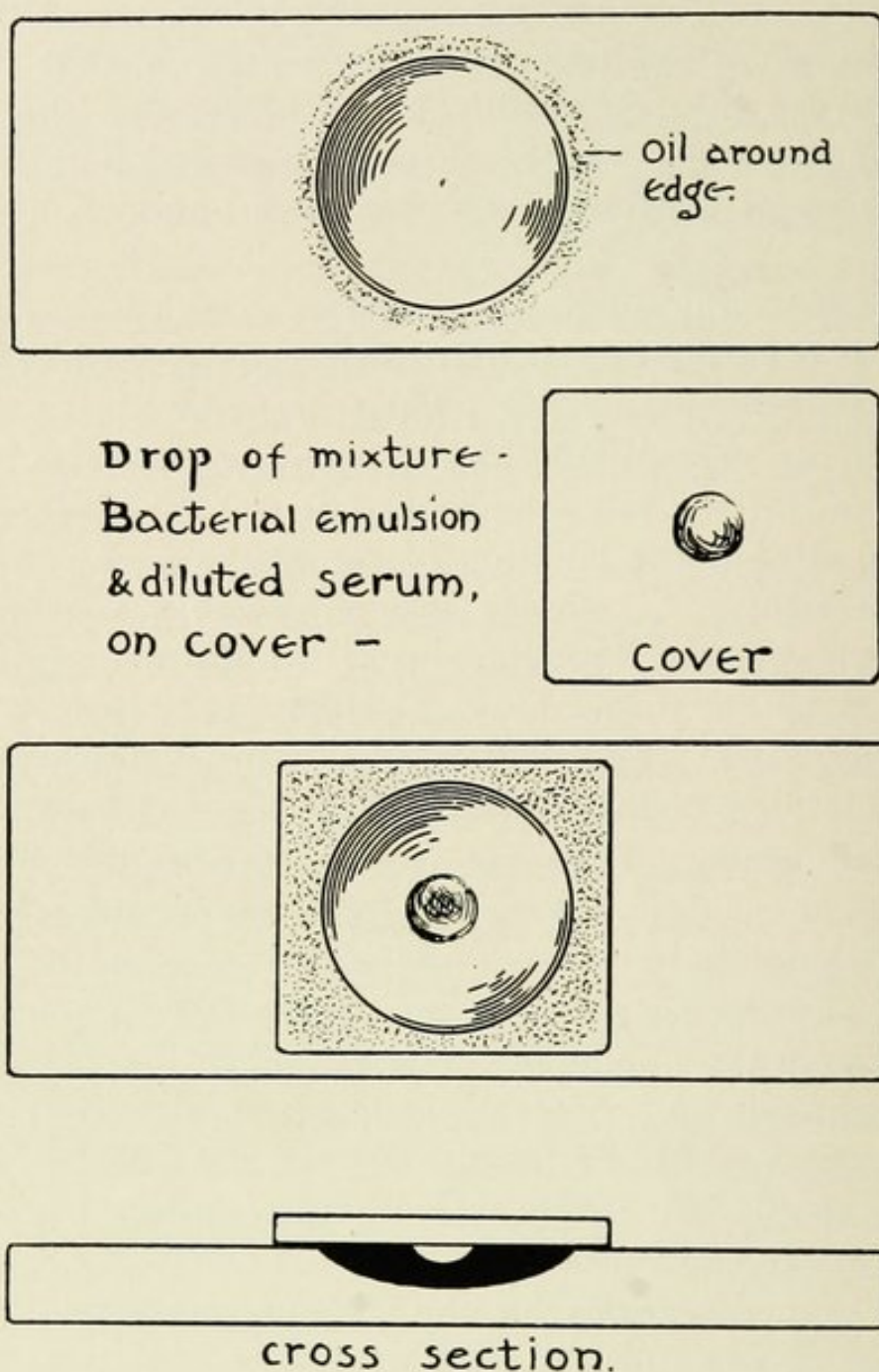


Fig. 11.—Method of mounting in the hollow-ground slide for microscopic agglutination tests and for determining motility of micro-organisms.

some laboratories the mixtures are made and stored in watch glasses.

Interpretation of Reactions.—The agglutination phenomenon consists of the clumping together of bacteria as a result of the

action of agglutinins in an antiserum. In order that the interpretation of the results may be correct it is necessary to examine the suspension without serum as well as immediately after the various dilutions of serum have been added. Microscopic agglutination tests for the typhoid fever diagnosis are usually observed after one or two hours of storage at the room temperature, while macroscopic tests are usually held for 12 to 24 hours for observation during the first hours of which they are usually kept in a water bath at 37° C. and after that are stored in the ice box. It is to be remembered that agglutination is a progressive process so that clumping increases in intensity as time elapses. When a suitable time after the mixture of bacterial suspension and serum dilution has elapsed, observations are again made.

When the microscopic test is made it is best to find the edge of the drop with the low power objective, then to swing in the high dry power (1/6 obj.) which should be brought down until it almost touches the cover glass, and then with the eye in position to bring the drop into focus by raising the tube. If these precautions are followed much loss of time, disappointment and breaking of preparations will be avoided. Under no condition are the observations to be made with the oil immersion objective (1/12). The positive reaction shows the bacteria thrown together in clumps. If the bacteria have been motile before agglutination, motion has ceased and there are no free bacteria. There are various degrees of reaction which will be considered later on.

To detect agglutination by the macroscopic test either the naked eye or a reading glass is used. The tubes containing serum in various dilutions are compared with the control tubes. The control tube should remain turbid. A positive reaction is manifested by small clumps or balls of bacteria which at first are uniformly distributed throughout the mixture but later fall to the bottom and leave a clear supernatant fluid. This latter condition is the most reliable basis for a diagnosis.

Before the results of agglutination tests are to be given diagnostic importance, various things must be considered and suitable controls must be made. Agglutination does not

always go on to the same degree: there may be only cessation of motility, there may be small clumps or there may be large clumps with occasional free bacteria or all the organisms may be united to form large clumps. While attempts have been made to define rigidly as to what shall be considered a positive reaction, it must always be remembered that the bacteria in contaminated or impure cultures cannot all be agglutinated by the agglutinins in a serum, that if there are more bacteria than the agglutinins can clump there will not be total agglutination and that the time required for complete agglutination varies. Whenever there is partial agglutination it is safest to regard the reaction as suggestive and to make further observations on subsequent days on the basis that if there are antibodies for the bacteria agglutination will become more marked and will occur in greater dilutions.

All tests must be accompanied by sufficient controls made for the following purposes:

1. To prove that the normal serum will not agglutinate the bacterial suspension used. Usually we assume that serum from a healthy person not having suffered with the disease should not cause agglutination in dilutions one-tenth as high as the patient's serum for which a positive reaction has been obtained. Thus if we are to regard as positive an agglutination of typhoid bacilli in dilutions of 1:50, normal serum should not agglutinate at 1:5.

2. To prove that the isotonic salt solution does not agglutinate as well as does normal serum. Pseudo-agglutination occurs at times with organisms grown for some time on artificial media and for this reason a salt solution control is made.

3. To prove that the agglutination is specific other allied organisms should be tested. This will be considered especially under the diagnosis of typhoid fever by the agglutination reaction.

When all of these precautions have been taken difficulties still arise. It at times is observed that there is no agglutination in dilutions of 1:20 and 1:50, while in all other dilutions up to 1:10,000 or higher there is clumping. These apparent discrepancies are not entirely explained. Some authorities believe

that in the lower dilutions there is a bactericidal action, while others assume that in fresh serum there are thermolabile substances having a greater avidity for the bacterial receptors than do the agglutinins, thus preventing agglutination. To avoid these disturbing features a test for diagnostic purpose should include various serum dilutions.

Real clumping of bacteria is at times confused with the thread reaction which occurs when bacteria grow in diluted serum. When this happens in macroscopic tests it is difficult to differentiate it from real agglutination. For this reason it usually is advisable to microscopically examine all specimens even when macroscopic tests are made. Under the microscope the thread reaction is easily detected for the bacteria stick together end for end and not in clumps as in true agglutination.

After all precautions have been taken and suitable control tests have been made the value to be assigned to the agglutination tests in a given case may still be doubtful. A negative reaction may result because the disease has not been of long enough duration to form agglutinins and a subsequent test may give a positive reaction. When a positive reaction occurs it must always be remembered that agglutination may be due to agglutinins acquired during a former illness, as the result of artificial immunization or that group, family or common agglutinins may be responsible for a positive reaction. The group or common agglutinins have already been considered on page 18.

The agglutination reaction is used especially for the diagnosis of typhoid fever but in other diseases as bubonic plague, Asiatic cholera and dysentery it is also of diagnostic value, while in glanders, pneumonia, meningitis and other diseases the reaction is used for the identification of the bacteria. Its value as a diagnostic measure in tuberculosis is not great.

Precipitation Test.—Precipitins are antibodies of the second order as described on page 18 having a single combining group which also contains a substance bringing about precipitation. If a specific antiserum is added to a germ free culture-filtrate of homologous bacterial species a precipitate is formed. This does not occur with normal serum nor for heterologous bacteria and albumin.

The discovery of the phenomenon was made for antibacterial sera by R. Krause in 1897 and for a sera for corresponding albumin solutions by Tsistowitsch, Bordét, Wolf and Wassermann. The relation of albumin from a single animal species to specific precipitins was first studied thoroughly by Nuttall, and its application in blood diagnosis for legal purposes was first pointed out by Uhlenhuth, Wassermann and Stern.

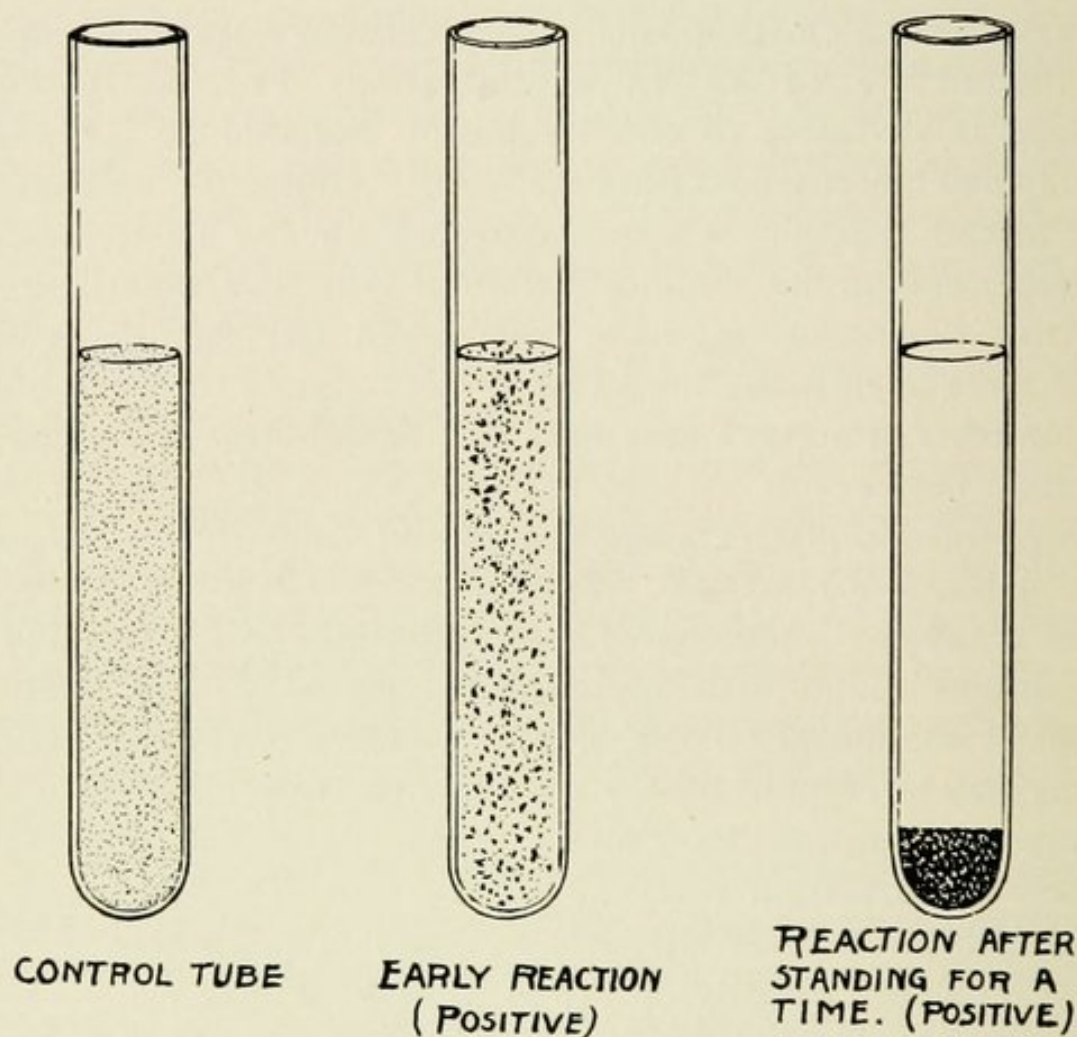


Fig. 12.—Precipitin test tube reaction.

The test is used relatively little for the diagnosis of bacterial infections but is used to some extent for the diagnosis of bacterial species and variety. The technique for the procedure for diagnosis of the infecting organisms according to Krause¹ is as follows:

The specific micro-organisms are grown on bouillon and after

¹R. Krause: Wien. klin. Wechschr., 1897, X, 736.

the growth has become profuse the culture medium is rendered germ-free by filtration through a Berkefeld, Chamberland or any of the filters that hold back all bacteria. This germ free bouillon is then put into small test tubes to which varying amounts of the diluted serum to be tested are added. A positive reaction is demonstrated by the formation of a powdery precipitate. In all cases control preparations must be made with the serum alone and with the filtrate alone. The precipitins, while specific antibodies, may show group and specific reaction. The reaction for the diagnosis of typhoid fever as advocated by Krause has given little satisfaction.

Bacterial precipitin tests are however much less frequently used than those for the identification of albuminous substances. Many albuminous substances on injection into animals produce in these animals corresponding precipitins which will precipitate the albumin used in the injection. The albumin in the milk of one animal calls forth especially the production of precipitins for the albumin of the milk of this species and variety of animals.

Uhlenhuth² has utilized the production of specific precipitins for the blood of man and animals as a means of determining the origin of blood stains. It has been found that drying blood or the mixture of blood for a long time does not interfere with the reaction. While the test may seem simple if it is to be used in legal cases the greatest care is necessary or the result may be invalidated.

The production of specific precipitating sera is of great importance. The specific animal that is to produce the anti-serum should be selected so as to be most distantly removed in species from the one whose albumin is to be precipitated. Of all laboratory animals the rabbit is most suitable for the formation of specific precipitating sera. For the production of serum which will cause a precipitate with human blood serum, rabbits are given five to six injections of eight to ten c. c. of human blood subcutaneously, intraperitoneally or intravenously. The interval between injections should be four to five days. Six days after the last injection the blood should be tested and

²Uhlenhuth: Deut. med. Wchschr., 1901, XXVII, 82.

if its precipitating value is high enough the animal is bled to death from the carotid artery or jugular vein. By this time all of the blood may be withdrawn but if only 40 to 50 c. c. are taken the animal may be kept alive. In forensic blood diagnosis part of the suspected material is first tested by such tests as Teichman's or by the aid of the spectroscope to determine whether the specimen really contains blood. To make the test reliable sufficient controls are necessary. Homologous and heterologous blood specimens are obtained from various animals. These as well as the blood in question are dried in the air, extracted with physiological salt solution, filtered through filter paper or even through a Berkefeld filter and then all solutions are diluted with salt solution until they are of a pale yellow color. Two c. c. of each solution are put in separate small test tubes and to each of these varying amounts of dilutions of the specific antiserum are added. Control tests are made by adding salt solution to tubes containing only blood solutions and to others containing only dilutions of the blood serum. All tubes must contain the same total amounts of fluid. This is accomplished by adding the right amount of physiological salt solution. All the tubes are now well shaken and placed in an incubator at 37 C°. From this time on the results should be noted every half hour. When precipitation occurs the mixture becomes flocculent and a precipitate falls to the bottom. In medico-legal cases this test should be accompanied by a fixation of complement test.

The question of specificity of the reaction is of the greatest importance. It has been found that precipitins are not entirely specific, thus the blood of a rabbit treated with the serum of the hen has precipitins not only for hen serum but also for that of pigeons. Nuttall was the first to thoroughly study this relationship. This need however not invalidate the results of the test if sera that have been previously tested are used and if the necessary controls are made.

Inasmuch as this test is of such great importance in cases in law it should only be undertaken by those skilled in serum diagnosis. As the precipitating power of serum can be preserved for some time by the addition of chloroform, phenol and

bi-chloride of mercury, it has been suggested that the sera used in legal cases should be under government control.

Precipitin tests are of value in the diagnosis of some infectious diseases, in the diagnosis of species and variety of some bacteria, and for the detection of the source of blood, meat, spermatozoa and albumin. It is impossible to differentiate the albuminous bodies of the same species. It was at first hoped that by precipitin tests some very valuable chemical problems pertaining to the nature of proteins might be solved, but it has lately been found that immunization to globulin yields precipitins for the serum-albumin as well as for the globulin in the homologous serum.

Bacteriolytic Tests.—Bacteriolysins are substances that are able to dissolve bacteria and occur in the blood and body fluid as a result of natural or artificial immunization. Normal serum of man and the animals contains bacteria-dissolving substances which are not specific but these are not usually referred to as bacteriolytic. Lysins cannot only be produced for bacteria but also for the blood cells and cells of organs of the body. These are known as bacteriolysins and cytolysins and are antibodies of the third order. Two substances, the specific antibody and complement, are necessary to produce lysis.

Bacteriolysins were discovered by Richard Pfeiffer and Issaëff who were engaged in the study of the immunity that can be produced artificially by injecting guinea-pigs with living or killed cultures of cholera spirillæ. They found that on injection of a dose of killed culture not in sufficient amount to kill the guinea-pig but only sufficient to produce a certain reaction from which the animal can recover, the animal's resistance is increased so that the ordinary lethal dose of living or dead cholera spirillæ will not be fatal. In studying the cause for this immunity they tested the power of the serum to destroy cholera organisms. By test tube experiments Pfeiffer was unable to detect an increase in bacteria-dissolving ability. But he observed that if a lethal dose of cholera spirillæ is injected into the peritoneal cavity of the living immunized guinea-pig and then parts withdrawn with a capillary pipette at intervals of one minute an interesting phenomenon can be observed.

At the end of one minute the spirillæ are still motile, those withdrawn a little later have lost their motility, at the end of ten to fifteen minutes they are broken up into granules and after an hour they have all disappeared. This phenomenon has been called Pfeiffer's reaction. To prove that it is dependent on the action of the blood serum of the immunized guinea-pig, he injected cholera organisms into the peritoneal cavity of normal guinea-pigs and coincidently or shortly after injected some serum from an immunized guinea-pig. Exactly the same phenomenon was observed as when cholera spirilla are injected into the peritoneal cavity of immunized guinea-pigs. Pfeiffer could not obtain this reaction in the test tube but Metchnikoff and Bordét of the Pasteur Institute using fresh immune serum found that the same phenomenon occurs in the test tube. Bordét made the important observation that the bacteriolytic power can be restored to immune serum which has lost its bacteriolytic power through age by adding very small amounts of normal fresh blood serum or peritoneal fluid. In this way he proved that two substances are concerned in bacteriolysis.

The examination of serum for specific bactericidal action may be either made by Pfeiffer's method or else *in vitro* by the method of Ehrlich and his pupils. The latter method does not give as constant results, requires absolute aseptic technique and considerable experience but is better adapted for purposes of diagnosing infection in man.

Pfeiffer's Method.—This method is especially adapted for the identification of certain bacterial species but at times is also of service in the detection of specific antibodies in the blood of patients suffering with infection.

For the diagnosis of bacterial species specific normal sera are necessary and are obtained from immunized laboratory animals, the rabbit being used most frequently. The serum is diluted as for agglutination tests but as the diluent usually bouillon is used instead of physiological salt solution. To one c. c. of diluted antiserum one loop of the fresh culture on solid medium to be tested is added and thoroughly mixed. Control tests are made in suspensions of bacteria diluted with one c. c. of sterile

bouillon and others to which one c. c. of only slightly diluted normal serum have been added. As soon as a mixture is made it is injected into the peritoneum of a medium weight (200 grams) guinea-pig. Usually after five minutes, after twenty minutes and after one hour a small amount of peritoneal fluid is removed by means of a capillary tube and examined in the fresh and the stained specimens. A positive reaction is demonstrated by the disappearance of bacteria in the peritoneal fluid of animals having received injections of the mixture of specific serum and bacteria, while the bacteria persist in the peritoneal fluid of the control animals. At times there is bacteriolysis in the lower dilutions of the specific serum and not in the higher ones, from this the titre of the serum is determined.

If the method is to be used for the determination of the presence of specific antibodies in the patient's serum, then 1 c. c. of dilutions of 1:10, 1:50, 1:100, 1:200 and 1:500 inoculated with one loop of the various cultures isolated from the patient and to be tested for their etiological importance are injected into the peritoneal cavities of guinea-pigs. After this small amounts are withdrawn from time to time and examined. The method requires many animals and is seldom used in this way.

Test Tube Method.—A uniform suspension of the bacteria in question is made in normal salt solution and equal amounts are put in a row of small test tubes. To each of these a small and equal amount of fresh guinea-pig serum is usually added and well mixed with the bacterial suspension. After this equal quantities of variously diluted serum containing, or supposedly containing, bacteriolysins are added and also well mixed. In some laboratories guinea-pig complement is not added, the freshly diluted antiserum being relied upon to furnish sufficient complement. In either case control tests consisting of tubes containing only bacterial suspension diluted with salt solution, bacterial suspension and complement, only complement, only antiserum, and one containing bacterial suspension, complement and normal serum are made. All control tests must be made up to the proper volume. The mixtures are then incubated for a time. In some laboratories all of the mixtures are

AN EXAMPLE OF A POSITIVE REACTION IS GIVEN IN THE FOLLOWING TABLE:

Tube No.	Bacterial Suspension.	Complement	1 c. c. Diluted antiserum	Salt Solution	Results 3 hours.
1	0.5 c. c.	0.5 c. c.	1:50	0
2	0.5 c. c.	0.5 c. c.	1:100	0
3	0.5 c. c.	0.5 c. c.	1:200	0
4	0.5 c. c.	0.5 c. c.	1:500	100
5	0.5 c. c.	0.5 c. c.	1:1000	10,000
Control	1.5	Immediate count, 20,000
1	0.5 c. c.	1.5	After 3 hours, 200,000
2	0.5 c. c.	0.5 c. c.	1.0	After 3 hours, 200,000
3	0.5 c. c.	0
4	1 c. c. 1:10 antiserum.	1 c. c.	0
5	0.5 c. c.	0.5 c. c. (1:10)	1 c. c. 1:10 normal serum	200,000

set up in duplicate or triplicate and the time required to produce lysis at certain dilutions is determined, while in other laboratories all determinations are made at the end of two to three hours. Whichever method is followed the entire contents of the tube or a definite amount of the mixture is transferred to a sterile Petri dish then covered and well mixed with molten agar. After this agar has hardened another coating is put on by pouring on about 5 c. c. more of molten sterile agar. The plates are then incubated, counts being made at the proper time.

If there is bacteriolysis there will be a reduction in the number of bacteria even to sterility in the plates containing the mixture of bacterial suspension, complement and antiserum.

Bacteriolysins due to immunization are quite specific although lysis occurs to some degree for closely related species of bacteria. The Pfeiffer reaction is generally specific enough for the identification of bacteria. His method also gives more constant and trustworthy results than does the test tube reaction.

Fixation of Complement Test.—The test is based on the observation that some antisera on mixture with homologous antigen (bacteria, bacterial extracts, extracts of organs in certain diseases) are able to bind or fix complement. The reaction is one dependent on the action of antibodies of the third order and complement. In 1901 Bordét and Gengou¹ showed that fresh guinea-pig complement added to cholera, plague, typhoid or anthrax bacilli, which had previously been treated with their specific antisera, is bound, absorbed or fixed. To determine whether complement is absorbed or fixed they made use of an earlier discovery by Bordét. Belfanti and Carbone had found that if a horse is injected with red corpuscles of rabbits and then later some of the horse serum is transferred to a rabbit the rabbit dies. Bordét while working on the bacteriolysis of cholera spirillæ tried to produce red corpuscle agglutinating serum and found that if he injected a guinea-pig with defibrinated rabbit's blood, the guinea-pig serum not only agglutinated the rabbit's erythrocytes but rapidly dissolved

¹Bordét and Gengou; Ann. de l'Inst. Past., 1901, XV, 289.

them. This solution Bordét¹ showed is due to the combination of the antibody of the antiserum and the complement present in fresh normal serum and antiserum. Complement may be fixed in solutions containing red blood corpuscles and their homologous antiserum or in solutions containing antigen and its homologous antibodies of the third order. By heat, age and so on complement in serum is destroyed and the serum is said to be inactivated. In their experiments Bordét and Gengou inactivated the antiserum and the antierythrocyte serum, then mixed the bacteria, antiserum and complement, allowed a time for their combination and complement fixation. If the bacteria and antiserum are homologous complement will be bound while if they are heterologous the complement will remain unbound. To detect whether or not the complement is fixed they then added red blood corpuscles and homologous antiserum. If the complement has been bound then the red blood corpuscles remain intact while if it is free the erythrocytes will dissolve.

This method was used by Malvoz² to show the presence of antibodies in the blood of animals inoculated with cultures of yeasts, by Moreschi³ to study the combination of complement and specific antiserum, and by Neisser and Sachs⁴ for the detection of blood in medico-legal cases. To Wassermann and Bruck⁵ however belongs the credit for making practical application of complement fixation for diagnostic purposes. Wassermann, Neisser, Bruck and Detre in 1906 adapted the method for the detection of syphilitic antibodies for which pure cultures of the causal micro-organisms were not available. Instead of the usual antigen they used extracts of organs of syphilitics. This special application of the fixation of complement by Wassermann and his pupils has so far overshadowed the work of Bordét and Gengou that these men are seldom referred to, the test being known almost entirely among the medical profession and laymen as "The Wassermann." Wassermann's method for the diagnosis of syphilis has been modi-

¹Bordét: *Ann. de l'Inst. Pasteur*, 1898, XII.

²Malvoz: *Ann. de la Soc. Med. Chir. de Leige*, 1901, XI, 275.

³Moreschi: *Berl. kl. Wochschr.*, 1905, XLIII, 1181.

⁴Neisser and Sachs: *Berl. kl. Wochschr.*, 1905, XLIII, 1388.

⁵Wassermann and Bruck: *Med. Klin. Berl.*, 1905, i, 1409.

fied in various ways, the one most frequently used being the method of Noguchi.¹ Fixation of complement tests for the diagnosis of specific antibodies are divided in two parts or systems. Of these the first consists of antiserum and antigen, while in the second are specific hæmolytic sera and the homologous red blood corpuscles. The test is used to determine whether a certain serum contains specific antibodies for a known antigen or whether the antigen is specific for a known antiserum.

To make the test the serum, inactivated by heating for a half hour to 56° C., is combined with the antigen. Then complement obtained fresh from the guinea-pig is added and the mixture is held at 37° C. for from one-half to two hours so that the antibodies, antigen and complement may combine. Next sheep red blood cells and specific serum dissolving sheep blood corpuscles are added. As the solution of red blood corpuscles by antibody in specific rabbit serum occurs only when complement is present, there will be no such solution if the complement has been fixed by the serum and antigen in the first part of the reaction. When no solution of the red blood corpuscles in the second part occurs then antigen and serum in the first part are homologous, while if there is solution then this is not the case.

The theory of complement fixation is shown graphically in Figure 13.

While this shows the theory of complement fixation actual performance of the test so it will give reliable results is not as simple. It is easy to see that most carefully made control preparations and accurate estimation of each of the five factors taking part in the test are necessary. If more complement is present than can be bound in the first part some will be left over to take part in the production of hæmolysis. The various control preparations will be referred to later. In the preparation of the different factors much care and study is necessary.

(a) **Serum to be Tested.**—This will vary somewhat according to whether the antigen is known and the presence of specific antibodies is to be determined or vice versa. If the correspondence of antigen to a known antibody is to be diag-

¹Noguchi: Jour. Exper. Med., 1909, XI, 392.

nosed then the serum is taken from an animal immunized in the usual way, while if the presence of antibodies is to be determined blood is drawn from the human or animal in question. The clear serum is inactivated by heating in a water bath to 56° C. for one-half hour. In the test varying amounts of different dilutions are used.

(b) **Antigen.**—If this is to be a bacterial extract the organisms are grown so that large amounts of culture may be obtained. Then an extract is made in a small amount of physiological salt solution to which 0.5 per cent. phenol or

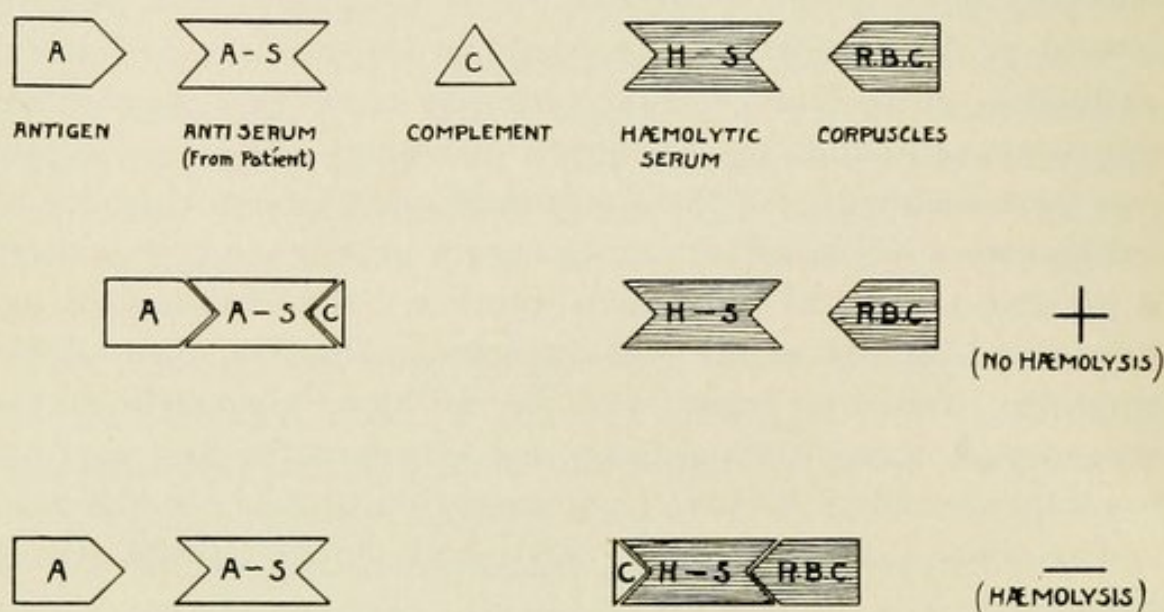


Fig. 13.—Graphic representation of the theory of the fixation of complement reaction.

toluol are added and the whole mixture is shaken several days in a mechanical shaker. After this it is centrifuged and the clear fluid drawn off. Of this fluid from 0.1 to 1 c. c. are used in the test. For the extraction of antigen from organs, salt solution and alcohol are generally used. For the preparation of antigen for the diagnosis of syphilis, see page 268.

(c) **Complement.**—Guinea-pigs, according to Noguchi and Bronfenbrenner¹ and others, furnish the best complement because this complement is most active and durable and is not as hæmolytic as that of other animals. It should not be used if

¹Noguchi and Bronfenbrenner: Jour. Exper. Med., 1911, XIII, 78.

drawn more than 24 hours before the test is made. As it is easy to obtain small amounts of blood directly from the heart of the living guinea-pig by means of the hypodermic syringe absolutely fresh guinea-pig serum is easily available. In complement fixation tests from 0.02 to 0.1 c. c. are used, the amount being determined and controlled by tests.

(d) **Hæmolytic Serum.**—Specific hæmolytic serum is best prepared by repeated injections of red blood cells of goats, sheep or man. For purpose of immunization about 10 c. c. of blood is drawn and mixed immediately with physiological salt solution containing 0.5 per cent. of sodium citrate or else immediately defibrinated by stirring. After this several washings of the corpuscles are made with physiological salt solution centrifuging between each washing. Then the washed corpuscles are injected into the peritoneum or under the skin at intervals of from four to five days. Several days after the third or fourth injections of corpuscles the serum should be tested to determine its hæmolytic value and if potent enough the rabbit is bled to death from the carotid artery, the serum allowed to separate out and then heated for one-half hour to 56° C.

(e) **Red Blood Corpuscles.**—These are obtained in the same manner as for immunization. Usually the corpuscles should be freshly obtained. In the test generally 1 c. c. of a 1 per cent. solution of washed red blood corpuscles is used.

The amount of the different factors to be used varies and usually has to be determined by investigation. Even after this has been done there are many possibilities that may invalidate the tests so that a number of control tests are necessary. The table on page 64 shows a fixation of complement test and the various controls.

It is not to be inferred that each of these control tests must be done for every test. One set of controls will do for any number of tests made with the same materials.

The principal application of the fixation of complement reaction has been made in the diagnosis of syphilis. It should be of value in all cases where the antibodies are of the third order, that is require and fix complement in exerting their

TEST OF SERUM FOR COMPLEMENT FIXATION.

	Serum, 1:100.			Antigen.	Complement, 1:10.	Salt Solution.	Hæmolytic sera, 1:100.	Corpuscles, 1%.	Hæmolysis.	
	Patients	Anti- serum.	Not Spec.							
—	0.2	—	—	0.5	0.5	0.8	1.0	1.0	+	Complement fixation negative.
—	0.5	—	—	0.5	0.5	0.5	1.0	1.0	—	Complement fixation positive.
—	0.8	—	—	0.5	0.5	0.2	1.0	1.0	—	Complement fixation positive.
CONTROLS.										
—	0.2	—	—	0.5	0.5	0.8	1.0	1.0	—	Complement fixation positive.
—	—	—	1.0	0.5	0.5	—	1.0	1.0	+	Complement fixation negative.
1	—	—	—	—	—	2.0	1.0	1.0	—	Test to insure inactivation of hæmolytic serum.
2	—	—	—	—	0.5	1.5	1.0	1.0	+	Test to insure activity of complement and presence of specific hæmolytic power of serum.
3	—	—	—	0.5	—	2.5	—	1.0	—	Test to determine that antigen will not dissolve erythrocytes.
4	—	—	—	0.5	0.5	1.0	1.0	1.0	+	Test to insure that antigen will not interfere with hæmolysis.
5	0.8	—	—	—	—	1.2	1.0	1.0	—	Test to determine that the serum in system I has been inactivated.
6	0.8	—	—	—	0.5	0.7	1.0	1.0	+	Test to determine that the serum in system I does not contain sufficient anticomplement to bind all the complement.
7	0.8	—	—	—	—	2.2	—	1.0	—	Test to determine that the serum in system I is not itself hæmolytic.
8	0.8	—	—	0.5	—	0.7	1.0	1.0	—	Test to determine that there is no hæmolysis when all but complement is present.

influence. The shortcomings of the method in the diagnosis of syphilis will be considered later. In all cases it is to be remembered that various chemicals affect complement and the other factors in the test, and that as Noguchi and Bronfenbrenner¹ have shown certain serum constituents lead to the disappearance of the complementary activity so that in addition to the controls on the test a number of other things must be considered before the result is to be regarded as a cardinal point in diagnosis.

It is important to remember that there are a number of non-specific hæmolysins as acids and alkalies, plant poisons (ricin and abrin), tetanolysin, staphylolysin, certain snake poisons, and that the normal blood of some animals dissolves the red corpuscles of certain animals.

Opsonic Index of Wright and Douglas.—In 1895 Denys and LeClef showed that serum is of importance in phagocytosis. Little attention however was given to the various observations on this subject until the work of Wright and Douglas was presented in 1903. Although Neufeld and Rimpau discovered probably the same substances as Wright and Douglas, these investigators are seldom referred to in the discussion of the changes in bacteria preparing them for ingestion by leucocytes. There are two definite reasons for the great prominence given to the work of Wright and Douglas: one is that they advanced and improved a technique for the determination of the amount of phagocytosis by means of which the opsonic index could be determined and the other is to be found in the widespread interest in the methods they advanced for active immunization of patients by the injection of killed cultures.

In 1902 Leishman presented a method for the quantitative determination of phagocytosis. He mixed equal quantities of patient's blood and bacterial emulsion, which he then incubated for a time in a moist chamber. After incubation he made cover glass spreads which he dried, fixed and stained. On these slides he counted the number of bacteria ingested by the leucocytes from which the average number per leucocyte was determined. This average he compared with the average per leucocyte ob-

¹Noguchi and Bronfenbrenner: Jour. Exp. Med., 1911, XIII, 92.

tained when normal blood instead of patient's was added to the bacterial emulsion. Leishman did not take into consideration the action of serum on bacteria or leucocytes but devised merely a "method of estimating phagocytic power."

Wright and Douglas in their work on the determination of the opsonic index modified Leishman's method to meet their theory on phagocytosis. According to their observation opsonin is a substance present in all serum. The amount varies in the different sera and as the amount varies so also will the extent of phagocytosis vary. To determine the phagocytic power it is necessary to have the three factors of phagocytosis in the mixture. These three factors are found in the blood serum, leucocytes and bacteria. A mixture of these three must be allowed to remain together for a definite period of time, after which the average number of bacteria taken up by the polymorphonuclear neutrophiles must be determined. This average number of bacteria per leucocyte is called the "phagocytic index." The phagocytic index obtained when serum from the patient is mixed and incubated with leucocytes and bacteria divided by the phagocytic index obtained when serum from a healthy individual is added to and incubated with a similar amount of the same emulsions of bacteria and leucocytes, gives the "opsonic index."

The technique here given is essentially the one developed by Wright and Douglas and demonstrated in New York City in 1906 by Wright and taught by his associate Dr. Ross. It is given somewhat in detail inasmuch as it is the one most generally used even though many modifications have been suggested and followed by different investigators.

The *serum* for the reaction is obtained as has been described on page 41, dried blood not being used in the test.

The *leucocytes* used in the determination of the opsonic index are usually obtained from the blood of supposedly healthy individuals, most frequently from the blood of the investigator himself. No particular importance is attached to the selection of the individual from whom the blood is obtained as long as he is healthy.

To obtain the leucocytes preferably the middle finger is

cleaned, congested and punctured in the same manner as was followed in the collection of serum. Ten drops of blood are collected in about 10 c. c. of a normal salt solution containing one per cent. of sodium citrate. The sodium citrate is added to keep the blood from clotting. The tube containing the mixture is then centrifuged at a low speed. When the speed exceeds 1,200 to 1,500 revolutions per minute the cells are too closely packed together and form clumps. The mixture is centrifuged until the corpuscles are thrown to the bottom of the tube and the fluid above is clear although it may be slightly straw colored. The supernatant fluid is then drawn off with a pipette. The corpuscles are washed free from serum and sodium citrate by again filling the tube with normal salt solution, mixing thoroughly and centrifuging until there again is a clear supernatant fluid. If the material in the centrifuge tube is now examined, one sees a clear fluid above and a red fluid in the lower part of the tube. The red fluid below however is not of the same shade throughout. The uppermost part consists of a grayish red layer—the leucocytes. These are layered above the red blood cells because of the difference in specific gravity of the leucocytes and red blood cells. It is from the gray layer that the leucocytes to be used in the determination of the opsonic index are obtained.

To obtain the leucocytes the clear fluid above is drawn off with a pipette and then with a clean pipette of about 1.6 m. m. inside diameter, the leucocytes are removed. To do this the pipette is firmly held in the hand, all of the air in the nipple is expelled and then as the pressure on the nipple is gradually released the open end of the pipette is held on the surface of the uppermost layer of grayish red color. This fluid will contain many red blood cells and also a relatively large number of leucocytes. After this fluid has been drawn into the larger part of the pipette the capillary end is sealed off. This fluid is called "leucocytic cream." Wright and Douglas state that in their experience there is no variation of the ability to engulf bacteria within the space of a few hours but that after three days the phagocytic power decreases to one-half or one-third of the original power.

The *bacterial emulsion* is made from such species or varieties of bacteria as may be of importance in the bacterial infection. In the selection of the particular culture to be used there are two sources—either the different cultures isolated from the lesion are used or else the same species and varieties of micro-organisms are taken from a stock culture. Usually living, freshly grown bacteria are used to make emulsions. The principal or practically only exception to this has been in the determination of the opsonic index for the bacillus of tuberculosis for which killed and old cultures of the organism are generally used to make the bacterial emulsion.

In all cases it is intended that the bacteria shall be well separated and suspended uniformly in salt solution. The technique for making the bacterial suspension varies with the different organisms, some of which it will be necessary to consider separately. The organisms for which the opsonic index is determined may be divided into three classes:

I. Many of the organisms belonging to this class grow on the ordinary media while for others media containing blood or other body fluids are necessary. The organisms occur singly or in pairs and when present in larger groups the cell aggregates can be easily broken up.

(a) *Mic. pyogenes aureus, albus* and *citreus*, *B. coli*, *B. typhosus*, *B. pyocyaneus*, and so on are grown on slant agar for twenty-four hours at 37° C. Various investigators, including Wright, have used four to five hour cultures of these organisms.

(b) *Mic. gonorrhææ* has usually been grown on hydrocele or human blood agar. Cole and Meakins obtained their cultures from growth on agar in which 0.5 c. c. of fresh blood had been added to 10 c. c. of ordinary agar. The age of the cultures used has varied from four to twenty-four hours incubation at 37° C.

(c) *Mic. meningitidis*, *Mic. pneumoniae*, and so on produce good growth on sheep serum agar. The cultures have usually been incubated at 37° C. from twelve to twenty-four hours.

To each culture of the proper age about ten drops of sterile normal salt solution are added. With a platinum loop the culture is washed off the media and with a thick walled capillary

pipette with the end broken off squarely the suspension is drawn into the pipette. The open end of the pipette is firmly pressed against a watch glass as indicated in Fig. 14. When this is done only a small crevice will remain between the end of the pipette and the watch glass. The bulb is now compressed and the bacteria suspended in the salt solution are forced out through the small crevices. This is done to break up the small clumps of bacteria.

II. The organisms belonging to this class grow on the same kind of media as do those belonging to the first group. The organisms of this group form cell aggregates that are not easily broken up. The streptococci are the most important organisms in this class. They grow in chains varying from two to thirty cocci. It is evident that a leucocyte may be able to engulf one or more cocci without being able to engulf a chain consisting of twenty or thirty cocci. The method for breaking up ordinary clumps will not suffice for breaking up the chains.

While observing the opsonic index for streptococci in erysipelas, the author¹ found it necessary to adopt some method to break up the long chains of streptococci frequently isolated from the lesions in erysipelas. This method consisted in the addition of 2 to 3 c. c. of sterile salt solution to each twenty-four hour culture on glycerin glucose agar.

After washing off the growth the emulsion was put into a small test tube containing sterile sea sand, the tube was sealed in a flame and shaken for one and one-half hours in the shaking machine. The sand and emulsion in the tube were centrifuged for one minute and the supernatant fluid drawn off. In this way short chains from two to four cocci were obtained.

Another method was devised by Wright of Harvard University. This method differs only from the one of Wright and



Fig. 14.—Pipette and watch glass for making uniform suspension of bacteria.

¹Schorer: Am. J. M. Sc., 1907, CXXXIV, 728.

Douglas in the substitution of a paraffine block for the watch glass. When the open end of the pipette is held against the paraffine the crevices are very small so that the chains are well broken up. This method gives very satisfactory results.

III. This class includes practically only one species, the bacillus of tuberculosis. The preparation of suspensions of tubercle bacilli is attended by numerous difficulties because this organism when grown on artificial media forms conglomerated masses. According to Wright and Douglas the living tubercle bacilli are heated to 100° C. before breaking up the clumps. Later Wright modified this technique by heating the bacilli to 100° C. on three successive days. The clumps, after this, are broken up in an agate mortar or in a watch glass, two or three drops of 0.1 per cent. salt solution being added at a time until two or three c. c. have been added. One-tenth per cent. salt solution is used in making this suspension because with greater concentration the bacilli are again clumped. Later Wright added 1.5 per cent. salt solution in making up the emulsion of tubercle bacilli because he found that this concentration is necessary to prevent spontaneous phagocytosis. When the heated bacilli are thoroughly rubbed and suspended in 1.5 per cent. salt solution, a homogeneous mixture containing but few clumps and many isolated bacteria results. This mixture is then centrifuged at high speed for about ten minutes. After this the supernatant fluid is drawn off and enough salt solution added to get the right concentration of the emulsion. While this method gives a fairly homogenous suspension of tubercle bacilli, still in an emulsion made in this way many of the tubercle bacilli are broken up. In determining the opsonic index it is necessary to count the number of bacilli taken up by the leucocytes and when there is fragmentation of bacilli it is necessary either to count each fragment as one bacillus or else to determine the fractional part of a bacillus. Either of these methods is most unsatisfactory.

Sellard and Jeans have emulsified the living tubercle bacilli in the same manner that has been used for the emulsification of other bacteria. After this they have killed the bacilli by exposing the emulsion to sunlight for a number of hours, ten

hours being sufficient to kill all tubercle bacilli present. In such emulsions they have gotten no spontaneous clumping, no fragmentation nor spontaneous phagocytosis.

Walker has recommended a method according to which Dorsett's egg medium is heavily inoculated with an actively growing culture of the bacillus of tuberculosis. After fourteen to eighteen hours of incubation at 37° C. salt solution is forced over the culture which is now rubbed off into the salt solution, the clumps being broken up with a platinum needle. The suspension is then filtered first through a loosely packed cotton filter and later through a filter made with scraped filter paper. Filtration is repeated until all clumps are removed after which the clump-free suspension is heated to 75° C. for twenty to thirty minutes.

The *strength of bacterial emulsion* most commonly used has a slightly opalescent appearance. It has been found that cloudiness may be absent or be only very slight and still the emulsion may contain too many bacteria. According to Wright's instructions the strength of the emulsion to be used is one which gives an average count of from five to eight bacteria per leucocyte when normal serum, leucocytes and the suspension of bacteria are incubated for the proper length of time. Walker has obtained better results with heavier suspensions of bacteria and diluted serum. It is not very difficult to make an emulsion of staphylococci, for after a bit of experience it can be determined by the naked eye whether the suspension of this species is heavy enough or not. The tubercle bacillus however presents greater difficulties it frequently being necessary to actually make a trial test for the bacterial emulsion. Simon and others have proposed the numerical determination of the number of bacteria per c. c. in the bacterial emulsion.

From the definition of the opsonic index it is evident that similar quantities of the same factors must be taken to make comparative mixtures. Furthermore according to the methods devised by Wright equal amounts of each factor are mixed together. When it is desired to use diluted serum equal volumes may still be used if the serum be diluted properly.

In order to get equal volumes Wright has made a capillary

pipette the walls of the capillary part of which are thick. The end of the pipette is broken off squarely. About a quarter or one-half inch from the open capillary end a mark is made with a soft wax pencil. This pipette is shown in Fig. 9. By means of a rubber teat which is attached to the large end of the pipette leucocytic cream is drawn into the capillary end up to the mark. Then the capillary end is withdrawn from the leucocytic emulsion and the emulsion in the tube is drawn about one quarter inch further up into the tube. After this the capillary end of the pipette is immersed in the bacterial emulsion which is taken into the tube up to the mark. The tube is withdrawn and again a small amount of air drawn into the capillary tube. After this the serum is drawn in, again taking the amount necessary to fill the capillary pipette to the mark. Walker has suggested that small quantities of serum and leucocytic cream be put into small tubes and that serum and leucocytes be taken only once from each of these small tubes. This is done in order to avoid carrying materials from one tube to another.

The capillary pipette now contains equal volumes of leucocytic cream, bacterial emulsion and serum. All three volumes are now forced out on a glass slide and drawn in and out with the pipette in order to mix the three parts thoroughly. When this has been done the mixture is drawn into the tube and allowed to come about half way up the capillary part when the open end is sealed off in a pilot flame of the Bunsen burner. Walker mixes the three factors in a small test tube taking precautions against air bubbles. Slides however have seemed better adapted especially because air bubbles are more easily avoided.

After sealing, the capillary pipette containing the mixture of leucocytes, bacteria and serum is incubated at 37° C. Realizing that the pipette containing the mixture will not assume the temperature of the thermostat if it is merely exposed to the air in the thermostat, Wright originated a so-called opsonizer. The length of time for which the mixture is incubated varies for the different organisms. It is to be noted that up to a certain limit the longer the period of incubation the more marked will be the phagocytosis. The incubation time must in some

cases be limited because of solution and agglutination of bacteria. The time of incubation varies from five to thirty minutes depending upon the species of micro-organism and the properties of the blood serum tested. In all instances the mixture containing patient's serum and that containing normal serum must be incubated at the same temperature and for the same length of time.

After incubation the nipple is removed and the capillary end of the pipette is broken off, the nipple is then replaced and the leucocytes, bacteria and serum are again thoroughly mixed on a glass slide and smears made. Wright and Douglas have devised an ingenious method to gather the leucocytes together in certain parts of the slide. The method of doing this is based

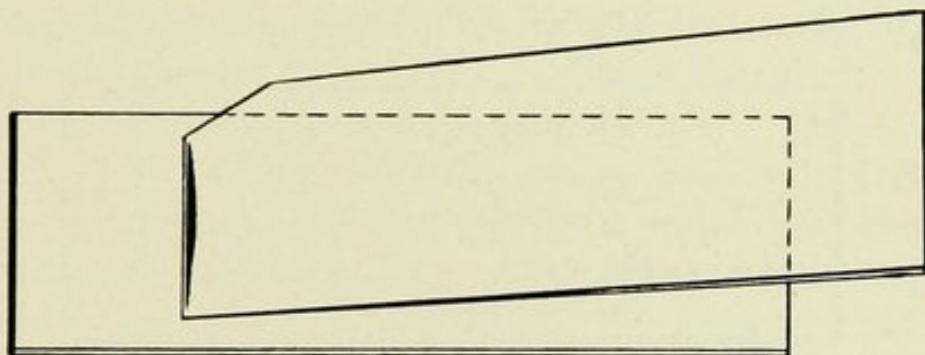


Fig. 15.—Spreader and slide for making smear for opsonic index determination.

on the difference in size of red blood cells and mononuclear and polynuclear leucocytes; the polynuclear and large mononuclear cells being largest in size.

The technique of Wright and Douglas is as follows: A slide is cleaned and slightly scratched by rubbing with jeweler's emory paper to slightly roughen the surface. On the left end of this slide a drop of the incubated mixture thoroughly mixed after incubation is placed. Then a slide with a smooth edge is made into a spreader by breaking off a corner. This is done so that the two margins of the spread shall be on the slide instead of running over the edges of the same. The narrowed end of the spreader is brought in contact with the slide and the drop of mixture allowed to run to its under edges. With the spreader held at an angle of 35° to 45° it is drawn over the

slide as is indicated in Fig. 15 and a smear having an outline as is indicated in Fig. 16 is made. The different steps taken in making the spread are essential to secure good smears. When the spreader is pressed firmly enough against the slide while it is being drawn over the same, the polynuclear leucocytes being larger than the red blood cells will slide out at the edge of the spreader or be drawn to the end of the spread. Care must be taken not to make the smear too thin.

After the smear has been made it must be fixed and stained for examination. Fixing and staining is usually accomplished by the ordinary blood stains, Leishman's, Wright's, Jenner's all giving good results except when the index for the tubercle bacillus is to be determined. The author has gotten more satisfactory results for organisms other than the bacillus of

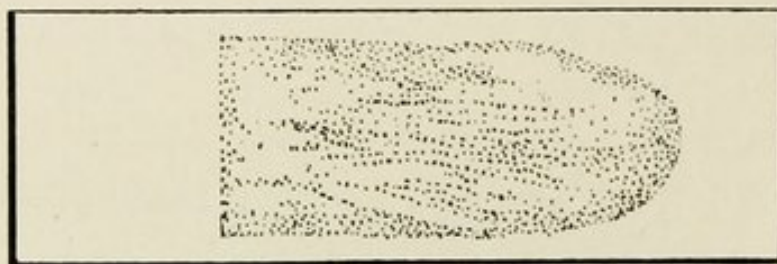


Fig. 16.—Smear for opsonic index determination.

tuberculosis by fixing the spread with methyl alcohol for one minute, washing off with water and then staining with Loeffler's methylene blue for three to five minutes. In staining slides on which the index for the tubercle bacillus is to be obtained the smears are first fixed in a saturated solution of bichloride of mercury which is then washed off with water. After this the slide is immersed in a jar containing Ziehl's carbol-fuchsin. The stain is heated by placing the jar in a heated water bath. After five minutes of staining in hot carbol-fuchsin the stain is washed off and the smear is decolorized in a mixture of 97 parts of alcohol and 3 parts of concentrated hydrochloric acid. Counter staining is done with a solution of one-half grams each of sodium carbonate and methylene blue in 100 c. c. of water. The counter stain acts rapidly and

has the advantage that if the slide be overstained in the process it can be easily decolorized with warm water.

The spreads are examined by first going over the slide with the low power of the microscope to determine whether the slide has been properly stained and also to find the part of the slide where the polynuclear leucocytes are most numerous. Usually these are most abundant near the margins and at the end of the smear. Under the high power of the microscope the number of bacteria engulfed by a definite number of polynuclear leucocytes is determined. The number of leucocytes examined varies. Wright based many of his determinations on examinations of twenty leucocytes. Most investigators however have counted the number of bacteria in fifty or more polynuclear leucocytes.

The average number of bacteria per leucocyte is spoken of as the phagocytic index. The opsonic index of Wright is determined by dividing the phagocytic index obtained when patient's serum is used with a certain leucocytic cream and bacterial suspension by the phagocytic index obtained when serum from the healthy individual is used with the same leucocytic cream and bacterial emulsion. This may be illustrated in a concrete case as follows: if the average number of staphylococci phagocytosed as determined by counting the cocci in fifty leucocytes is eight when the patient serum is used and ten when normal serum is used, then eight-tenths (0.8) is the opsonic index.

Wright's publications on opsonins, the part they play in immunity and the method of determination of the opsonic index lead to much investigation on these subjects. Many investigators followed Wright's methods closely while some have followed what they supposed were Wright's methods. The first reports of work done by investigators other than Wright and his pupils agree strikingly with the results obtained by Wright. Later results however were not as favorable. For a time want of personal skill and ability, improper methods of work and lack of ability to manipulate were supposed to account for results which did not agree with those obtained by Wright. Numerous investigators however after having received in-

structions from Wright and his pupils on the methods of determining the opsonic index seriously questioned his methods. This list of investigators includes Park, Simon, Baldwin, Cole, Moss, Potter, Bolduan, Walker and many others. On the other hand there are numerous investigators who have not questioned the technique and the results obtained by following the methods of Wright. These investigators followed principally the opsonic index in the various bacterial diseases and made efforts to determine the nature of opsonins and their importance and behavior in these diseases.

The mechanical part of the technique of Wright has probably been less criticised than any other part of his method. Various investigators have reported their technique in full and some at least have shown that they have not interpreted Wright's explanations of the same correctly while others have modified the technique in order to give greater accuracy to the method of the determination of the index. Before the opsonic index as determined by any method can be accepted some of the difficulties will have to be overcome.

Even after the technique has been perfected the importance of the opsonic index as a diagnostic measure or as a guide in the process of immunization must be established.

According to Wright the opsonic index of a normal individual will vary from 0.8 to 1.2. To obtain the true amount of opsonin in normal serum he pools equal quantities of sera from several normal individuals. However after it has been established that a healthy individual has an opsonic index of 1.0 as determined by estimation with pooled normal sera he uses serum from this individual as normal serum. Wright's own determinations from which he concedes that there are variations in the amount of opsonin present in the blood of healthy individuals discredit the accuracy of the method of determining the opsonic index and the value of the index. If the opsonic indices of two normal individuals are 0.8 and 1.2 respectively on the same day as determined by comparison with pooled normal serum, then as compared to each other their opsonic indices vary from 0.66 to 1.5 respectively. It is thus evident that from Wright's concession the index of a normal individual may

vary from 0.66 to 1.5 when one serum is used as a control for the other.

Indices for normal serum as actually determined will not even vary within these limits. The author some time ago reported¹ results on a series of ten specimens of blood from ten healthy adults studied to determine the differences in opsonic power in healthy individuals. The leucocytes were washed twice with 0.85 per cent. salt solution. The number of staphylococci taken up by 100 polynuclear leucocytes was counted in two preparations separately incubated. The phagocytic indices as determined by two workers A and B are as follows:

Source.	A	B
N	9.48	7.63
J	9.16	5.20
B	8.75	6.80
McL	7.27	4.37
Z	6.63	5.12
C	6.60	5.37
S	5.89	4.82
O	5.84	4.57
H	5.49	6.03
Ns	4.91	4.37

The great error introduced by this method becomes evident upon the determination of the opsonic indices when each serum is used for comparison with all of the others. In the series A the normal opsonic index varies from 0.52 to 1.93, while in series B it varies from 0.57 to 1.75. Moreover the indices obtained by the two workers A and B vary considerably for the same sera.

Bolduan has reported determinations of the opsonic index of normal individuals and finds that as far as the bacillus of tuberculosis, staphylococci and streptococci are concerned there is marked variation not only in different individuals but also from day to day. Moss has found opsonic indices for staphylococci to vary from 0.18 to 0.56 in normal rabbits.

A number of experiments have been made in which the same serum has been tested a number of times with the same leucocytes and bacterial emulsion. Bolduan reports opsonic index determinations for a single serum which vary from 1.05 to 1.46. The writer has reported determinations made by taking five

¹Schorer: Amer. J. M. Sc., 1907, CXXXIV, 728.

specimens of blood from the five fingers of the same hand of a healthy adult. The leucocytes used for the determination were washed twice in 1.5 per cent. potassium citrate in normal salt solution. The phagocytic indices found for *Mic. pyogenes aureus* are shown in the table.

	1st 100 leucocytes counted.	2d 100 leucocytes counted.	Phagocytic index.
Specimen 1.....	166	196	1.810
Specimen 2.....	176	183	1.795
Specimen 3.....	198	199	1.985
Specimen 4.....	185	175	1.800
Specimen 5.....	230	190	2.100

If the indices for each specimen of blood be compared with all the others, the opsonic index as determined by counting the number of cocci in two hundred cells will be found to vary from 0.85 to 1.22.

It thus seems evident that if normal serum varies as much as has been found, the variations from the normal unless marked can indicate but little. The most that would seem warranted is to determine whether a serum is "high" or "low" in opsonin content as the case may be.

Walker has found that with many species of bacteria undiluted normal serum will opsonize so many bacteria in a heavy suspension that the leucocytes will contain too many microorganisms to make counting possible. If the bacterial suspension be made less heavy fewer bacteria will be taken up by the leucocytes but under this condition the serum will not be exhausted of its opsonin and part will be lost in the estimation of the opsonic index. A serum containing much less opsonin may opsonize just as many bacteria in a light bacterial suspension as a serum containing more opsonin. "When thin suspensions of bacteria for which much opsonin exists in the serum are used it generally happens that both the sera sensitize all the bacteria so that the work, if accurately done, will produce equal phagocytic indices for both—in other terms, an opsonic index of unity—regardless of the real relation of the serum. Phagocytic indices proportional to the sera tested may readily be obtained by diluting all the sera equally to a sufficient degree and using with these diluted sera a thick bacterial sus-

pension." (Walker.) For some bacteria as *B. typhosus*, certain strains of streptococci and tubercle bacilli he does not recommend the dilution of serum while for staphylococci, some strains of streptococci and tubercle bacilli and for *B. coli* he does. Walker's results obtained by dilution of serum differ from Moss' who compared the opsonic index in various dilutions of serum and found that the index obtained in one dilution is not proportional to that obtained in all the other dilutions. Simon, Lamar and Bispham have shown that by the dilution of some sera there often is a rapid exhaustion of phagocytic power of the leucocytes. They observed that undiluted pig's serum manifests a most intense opsonizing effect on staphylococci but that this action diminishes markedly upon dilution of the serum. Human serum on the other hand though not having as marked an initial opsonizing power will retain this power on much greater dilution. They have further found that in human beings who are supposedly in good health the phagocytic power in concentrated serum will in some cases diminish in proportion to the degree of dilution while with the serum of other individuals more rapid exhaustion takes place.

Most investigators have experienced difficulty in obtaining suitable suspensions and Wright himself has changed his technique for making bacterial suspensions from time to time. The difficulties that have been experienced are of two kinds: those dealing with the strength of the bacterial suspension and those concerning the condition of the micro-organisms in the suspension. In some laboratories the bacterial emulsions have been made of such strength as to get a definite phagocytic index with normal serum. In most cases it is desirable that the phagocytic index for normal serum be between six and fifteen. Simon has found that when the emulsion contains between 666,000 and 2,000,000 micro-organisms per cubic millimeter the best results are obtained. Recently Walker has emphasized certain facts that must be taken into consideration in determining the strength of bacterial emulsion. If the suspension does not contain enough bacteria to exhaust all the opsonin in the strongest serum a certain amount of opsonin will be lost in the estimation of the opsonic index. In order to get the right

strength of bacterial suspension Walker tests the same with serum diluted 1 to 30 and 1 to 15. If the 1 to 15 dilution of serum shows a phagocytic index twice as great as that obtained in the 1 to 30 dilution then enough bacteria are present in the suspension. To such a suspension he adds more of the culture because having more bacteria than are absolutely necessary will not change the opsonic index but will certainly furnish enough bacteria to exhaust the serum of its opsonin. Only with the staphylococci will a too heavy suspension affect the true index. This Walker believes is due to the action of a product of bacterial growth. To overcome the excessive phagocytosis when heavy suspensions of bacteria are used Walker dilutes the serum.

In examining slides for the determination of the opsonic index difficulty is encountered because of solution and clumping of the bacteria. These difficulties are apparently dependent upon the particular culture used but to a greater extent upon the agglutinins and lysins present in different sera. To overcome this difficulty young cultures are preferred because they stain better than do old ones. To avoid agglutination and lysis the serum is in some cases heated to 55° C. or 65° C. The clumping of bacteria in opsonic index preparations is given little attention by Wright. Other investigators have however regarded clumping of bacteria as responsible for the occasional enormous differences of the indices determined.

To the source of the leucocytes Wright attaches little importance while most other investigators have aimed to obtain leucocytes from the blood of apparently healthy individuals. The work of Peterson, and Hiss and Zinsser indicates that the leucocytes contain substances that are of importance in immunity and that these substances vary in the leucocytes of normal and immunized animals. For this and other reasons it seems advisable in all cases to obtain the leucocytes from the blood of normal individuals. In all work on the determination of opsonic indices it has been found that there are great differences in the number of bacteria taken up by the individual leucocytes. Moreover great differences have been found in the phagocytic index as determined by different investigators examining the

same slide. Even the same investigators have obtained markedly different phagocytic indices on the same slide. Cells which are apparently normal as far as can be determined by staining and which may contain an average of from 5 to 8 bacteria per leucocyte will in some cases phagocyte from 20 to 30 micro-organisms while others may take up none. This difference in the number of bacteria taken up by individual leucocytes has been supposed to be due to differences in the leucocytes themselves and to clumps in the bacterial emulsion. In one of the early contributions on the subject of opsonic indices Simon, Lamar and Bispham recommend that in making the spread of the mixture after incubation, the spreader slide be merely kept in contact with the blood without touching the lower slide for "otherwise it may happen that most of the leucocytes containing organisms are carried to one end while only the empty cells are found in the intervening space." In the laboratories of the Johns Hopkins Hospital, Cole, Moss, and Jeans and Sellard made determinations of phagocytic indices determined in different parts of the slide. It was found that the leucocytes collecting near the edge of the smear contain decidedly more bacteria than those toward the center. By dividing a slide into three zones they found that at the end of the smear the leucocytes contain more bacteria than in the first and middle zones. To explain these differences these men assume that the polymorphonuclear leucocytes containing the largest number of bacteria are so increased in size that they are drawn to the end of the slide while the smaller ones drop out earlier. These results show that all the leucocytes should be examined and not only those at the end of the spread as was advocated by Wright.

In determining the opsonin content of blood Simon has proposed that the percentage of phagocytosing leucocytes with patient's serum be compared with the percentage with pooled normal blood serum. The value obtained for pooled normal serum he called 1.0 and the index determined in this way he called the percentage index as contrasted with Wright's bacillary index. Strong in his early experiments with antiplague serum determined the index according to Wright's method.

Later however he substituted this method by one in which the highest dilution of an immune serum which gives a marked phagocytosis is compared to the same dilution of a normal serum. If with a certain dilution of an immune serum marked phagocytosis is observed while the same dilution of a normal serum gives only slight phagocytosis, the immune serum is regarded as having an increased opsonin content. Even on this method Strong places little reliance.

Wright's method of the determination of the opsonic index has yielded such inconsistent results that more work on this subject seems justifiable. It must be remembered that the opsonic index is of no value whatever unless carried out with the greatest care and by someone who has had considerable experience. Modifications of Wright's technique have arisen with too much rapidity to make possible any definite rule for the determination of the opsonic index. However there are only few modifications that have made it possible to get more reliable determinations than did Wright.

III. Hypersensitiveness, Altered Susceptibility, Allergy.

For many years it has been known that following attacks of certain acute infectious diseases there remains a certain immunity. Early in the eighteenth century this led to experimental artificial immunization against smallpox. This marked the beginning of intentionally infecting and injecting into the animal certain substances so as to induce an immunity. In his experiments on smallpox vaccination Jenner observed: "It is remarkable that variolous matter when the system is to reject it should excite inflammation on the part to which it is applied more speedily than when it produces cowpox." This statement he based on the early inflammation following smallpox vaccination in persons and animals already immune. Magendie in 1859 observed that repeated injections of egg albumin at times give rise to serious symptoms. In 1891 Koch observed that tuberculin is almost without action in the tubercle-free animal while it is highly toxic to the tuberculous body. He further observed that tuberculous guinea-pigs into which tubercle bacilli are again introduced subcutaneously

react in a special manner for very soon an active inflammatory process develops at the site of the second inoculation. This eventually brings about the expulsion of the bacilli with the slough and is followed by complete healing. The tubercular body reacts in the same manner to dead or living tubercle bacilli. On this the various tuberculin tests for diagnosis are based. In 1898 Richet and Herricourt showed that repeated injections of eel serum into dogs instead of producing an immunity actually produces more susceptibility. Portier and Richet in 1902 determined that poisons of certain actinians when injected in sub-lethal doses produce after ten days a hypersensitiveness to another injection of a sub-lethal dose of the same poison. Arthus in the next year reported that if rabbits receive repeated injections of horse serum at intervals of six to eight days the second injection is likely to produce severe symptoms and even death. Theobald Smith at this time called attention to the sudden death of guinea-pigs after the second injection of diphtheria toxin-antitoxin mixtures. Soon after this time appeared the work of Otto, Rosenau and Anderson, and von Pirquet and Schick all relative to "serum disease" "anaphylaxis" and "allergy."

The application of altered sensibility for the diagnosis of specific infection began with Koch's researches with tuberculin in tuberculosis. In more recent years it has received a new impetus mainly because of the work of von Pirquet in 1903 and 1904, which led him to realize that only those previously vaccinated showed within 24 hours after a subsequent vaccination the "early reaction." From this observation which had been made by Jenner but had not been noted especially, von Pirquet was led to the discovery that a drop of tuberculin injected into the skin of a tuberculous individual showed evidences of hypersensibility, a local reaction occurring and remaining confined to the place of injection. Based on his investigations he concluded that "allergy in the form of the early reaction following cutaneous injection can be used for diagnostic purposes in vaccinia, variola, tuberculosis and probably a number of other infectious diseases." Then there followed the skin reaction of von Pirquet, the conjunctival reaction of Calmette (first sug-

gested by Wolff-Eisner), then the percutaneous or ointment method of Moro, the intracutaneous reaction of Mantoux and Roux—all concerned with the diagnosis of tuberculosis. Following von Pirquet's prophecy, Chantemesse has developed an allergy reaction for the diagnosis of typhoid fever, Meirowski, Wolff-Eisner, Neisser and Bruck and others have attempted to diagnose syphilis by sensibility reactions to extracts of syphilitic tissues, Noguchi has developed his luetin reaction for the diagnosis of syphilis and Irons has shown there is an allergy in infections with *Mic. gonorrhææ*. In recent years some of the so-called idiosyncrasies to common foods have been found to be due to allergy and appropriate skin tests have been made to determine such altered reactions.

The various theories advanced to explain allergy will be considered in another chapter. Anaphylaxis occurs as an incident in the course of immunization to protein substances, the sensitized individual or animal apparently splitting up protein so rapidly that toxic substances are so quickly set free as to produce symptoms and signs. Allergy appears from four to twelve days after the sensitizing substance has entered the body and persists for periods varying from a few weeks to years.

For diagnostic purposes small amounts of substance are injected subcutaneously or cutaneously, are rubbed into the skin or dropped on the mucous membrane especially of the eye. The details of technique employed vary in the hands of the different observers. Subcutaneous and cutaneous injections or abrasions as in smallpox vaccination should be made on parts of the body easily accessible for examination and not subjected to rubbing with clothes. In making cutaneous tests control tests should always be made with the non-specific parts of the specific substance used in the test. This must be done to distinguish the non-specific cutaneous reaction due to mechanical irritation and the reaction produced by the culture media, glycerin, salt solution and so on used in the preparation of the specific substance from the reaction due to the specific agent itself.

The reactions in the tests vary. The diagnostic signs fol-

lowing suitable subcutaneous injections are usually fever, malaise, loss of appetite, hyperæmia about the lesions and at times difficulty in breathing. If there is especial hypersensibility or large doses are injected the symptoms may be severe and death may even result. Cutaneous and mucous membrane reactions are evidenced by hyperæmia, congestion and vesicle or pustule formation, constitutional symptoms seldom following proper cutaneous or mucous membrane injections or instillations. Comparison with similar injury to the skin not treated with the specific substance must always be made. Usually there is no difficulty in recognizing a positive reaction. The diagnostic symptoms and signs usually appear after twelve to forty-eight hours, come to their height rather quickly and then disappear.

Allergy reactions are of great assistance in the diagnosis of tuberculosis, are now used to some extent for the diagnosis of syphilis, typhoid fever and gonococcus infections and will quite certainly be used in the diagnosis of other conditions. Unfortunately there are some drawbacks to this means of diagnosis. The reaction is not indicative of the severity and extent of the infection. Allergy is not entirely specific to the distinct proteins nor is it possible in most cases to get antigen that contains only the specific substance. Hypersensibility furthermore may be transmitted from mother to child so that the child may give the reaction due to the allergy of the mother. These tests as most other specific tests should only be relied upon as cardinal factors in the diagnosis.

IV. Chemical and Empirical Tests.

Chemical studies of the body tissues, fluids, excretions and secretions in health and disease have led to various tests of value in the diagnosis of disease. In the last few years attempts have been made to utilize the results of chemical examinations for the diagnosis of specific causes of disease. Most of the tests advocated have however failed to determine the etiological factor and now are used principally as links in the chain of evidence on which the diagnosis is based. Many of the tests must be classed with symptoms and signs of the disease and must be given no greater consideration than these

Only a few of the more recently advocated and partially specific tests are described here.

I. NOGUCHI'S BUTYRIC ACID TEST.—Noguchi observed that syphilitic antibody is contained in or precipitated with the globulin of the blood and cerebrospinal fluid and that the globulin fraction is usually increased in syphilis. Moreover the globulin fraction is increased earlier than the antibody and this increase is more constantly observed in latent syphilis than is the presence of demonstrable antibody. The reagents for the tests are a 10 per cent. butyric acid solution in physiological (0.9 per cent.) salt solution and a normal sodium hydrate solution.

To make the test cerebrospinal fluid free from blood is necessary. Of this two parts are mixed with five to ten parts of the butyric acid solution and boiled for a brief period. After this one part of the sodium hydroxide solution is added and again boiled a few seconds. The quantities usually used are 0.1 to 0.2 c. c. of cerebrospinal fluid, 0.5 c. c. of the butyric acid solution and 0.1 c. c. of the normal sodium hydrate solution. Increased globulin is indicated by precipitation with a clear supernatant fluid while normal fluid remains clear or at most is only cloudy or opalescent.

To test blood serum one part of hemoglobin-free serum is mixed with nine parts of a half-saturated solution of ammonium sulphate and the precipitated globulin centrifuged out well. The globulin fraction is then weighed and dissolved in ten parts of physiological salt solution. One part of this dissolved globulin is mixed with an equal amount of the 10 per cent. butyric acid solution. Noguchi uses 0.5 c. c. of serum, 4.5 c. c. of ammonium sulphate solution, 5.0 c. c. of physiological salt solution, and of the dissolved globulin he mixes 0.5 c. c. with 0.5 c. c. of the butyric acid solution. Greenfield¹ has modified this test, using 2.0 c. c. of fluid and determining the albumin in a graduated centrifuge tube. Normal fluids give readings of 0.005 to 0.02 c. c. while cerebrospinal fluids in syphilitic disease give readings up to 0.6 c. c.

Noguchi has found the reaction positive in the cerebrospinal

¹Greenfield: *Lancet*, (Lond.), 1912, II, 685.

fluid of syphilitic and parasyphilitic affections and also in all cases where there is an inflammation of the meninges caused by *Mic. intracellularis*, *Mic. pneumoniae*, *Bact. influenzae*, *Bact. tuberculosis* and so on. The blood serum reaction he has found positive in cases of syphilis while there was no reaction if the blood serum came from patients not suffering with syphilis. While the test does not give a diagnosis of the etiological factor it is of value in detecting inflammatory conditions in which the cerebrospinal fluid may be clear as in tuberculosis and syphilis.

II. COBRA VENOM TESTS.—The hæmolyzing action of cobra venom has long been known. Cobra venom however does not dissolve the red corpuscles of all animals in a like manner; the corpuscles of man, dog, horse and guinea-pig are dissolved directly but for the solution of those of the sheep and goat an activating substance is necessary in addition to the cobra venom. Much and Holzman found that the blood serum of patients suffering with mental disorders inhibits the hæmolysis of red corpuscles and they concluded that in blood from such persons there is an inhibiting substance derived from degenerated nerve tissue. Weil has observed that the red blood cells of syphilitics are more resistant to cobra venom than those of healthy individuals. The cause for this resistance is not known but it is supposed to be due to the decreased lecthin content of the red corpuscles of syphilitics. To make his test he mixes fresh blood from the patient with a sodium citrate salt solution and by centrifugalization and washing with salt solution removes all of the patient's serum from the corpuscles. Then an accurate 4 per cent. suspension of these corpuscles is made. A cobra venom solution is made from dried venom in a 0.05 per cent. solution in a 0.9 per cent. salt solution. The potency of the venom is obtained by testing it with erythrocytes from known healthy and syphilitic persons. In making the test various dilutions of the venom are used, the dilutions being determined from the potency tests. One c. c. of the corpuscle suspension and one c. c. of the venom dilution are mixed and incubated at 40° C. for one hour after which observations are made. The mixtures are kept in the ice chest over night when the final observations are made. The lower dilutions should

not hæmolyze while the higher ones should. Higher dilutions of venom are more necessary for testing children's corpuscles than those of adults.

The venom test of Much and Holzman has little diagnostic value while the Weil test is considered to be of much value especially in secondary and tertiary syphilis.

III. NONNE'S AMMONIUM SULPHATE OR PHASE I TEST.—This test is made by bringing together equal parts of cerebrospinal fluid and saturated ammonium sulphate solution. This solution is saturated while hot but before the test is made it is allowed to cool. Cloudiness should appear immediately or within three minutes if the test is positive. A positive reaction is regarded by Nonne as evidence of an abnormal spinal fluid which may or may not be due to syphilis. It has been found in most laboratories that the test is of value in the detection of organic disease of the nervous system but Apelt in his series found the reaction negative in a large percentage of cases of syphilis in which the nervous system was unaffected. On the whole the reaction is negative when the increase in globulin is slight.

IV. ALBUMIN TEST OF SPUTUM.—Various attempts have been made to devise means for diagnosis of pulmonary tuberculosis when it is impossible to find tubercle bacilli in the sputum. Of these the test for albumin in the sputum has been given much attention recently. To remove the mucin a weak (three per cent.) solution of acetic acid is added to the sputum. After thoroughly mixing, the mixture is allowed to stand for ten to fifteen minutes when it is filtered through filter paper. To the clear filtrate a few more drops of acetic acid are added to be certain that all of the mucus has been removed. The clear fluid is then boiled during which time crystals of salt are dropped in. If albumin is present there is clouding or precipitation.

Levy-Valensi¹ found albumin in the sputum in all cases of chronic or acute pulmonary tuberculosis and believes it offers an earlier method of diagnosis than does the bacteriological examination. Fullerton² on the other hand states that in a

¹Levy-Valensi: *Med. Press and Circ.*, (Lond.), 1912, XCIII, 3810.

²Fullerton: *Glasgow M. J.*, 1912, LXXCIII, 1.

small proportion of cases of pulmonary tuberculosis there is no albumin and that furthermore there is albumin in the expectoration of patients suffering with bronchitis and pneumonia during the febrile stage, in pneumonia during the resolution stage and in bronchiectasis. He concludes that large amounts of albumin in the expectoration suggest tuberculosis except in the febrile stage of bronchitis and in pneumonia and bronchiectasis. Fishberg¹ believes that in cases of tuberculosis in which albumin was present in the sputum and has disappeared cicatrization of the pulmonary lesion has occurred and when albumin reappears after a negative reaction has been obtained for some time exacerbation is suggested. The test while not entirely reliable in diseases of the respiratory system and of limited value in the diagnosis of pulmonary tuberculosis of the lungs is of value in indicating the presence of some pathological condition in which the lungs are affected.

V. Inclusion Bodies.

There are a number of infections and communicable diseases which in all probability are caused by some specific micro-organisms which have not yet been seen or certainly identified. In a number of these diseases examination of the tissues or body fluids has revealed the presence or existence of what have been called "inclusion bodies." With the discovery of each of these it was hoped that the etiological factor in the disease had been found or that at least a more certain means of diagnosis had been developed. These bodies must always be searched for in the proper tissues or fluids and be properly stained. Of all the inclusion bodies the Negri bodies found in rabies are probably the most specific and have been of most importance in diagnosis. Inclusion bodies have also been found in smallpox, scarlet fever, and so on. In no studies so far undertaken have they been proven to be etiological factors.

VI. Filterable Viruses.

Filterable viruses cause some of our most important diseases in man and the animals. They are micro-organisms which will

¹Fishberg: Arch. of Diag., 1912, V, 220.

pass through the pores of filters too small for ordinary bacteria. The two principal filters meeting these requirements are the Berkefeld filter made of diatomaceous earth and the Chamberland filter made of unglazed porcelain. The first of the filterable viruses was discovered in 1898 by Loeffler and Frosch who found that the causal factor of foot and mouth disease will pass through fine porcelain filters. Since then some thirty filterable viruses have been discovered. By many the micro-organisms passing through filters are said to be ultramicroscopic but while few satisfactory visible demonstrations of these organisms have been made, new applications of the dark-field method of illumination, the ultramicroscope and the ultraviolet rays apparently offer the possibility of demonstrating some of these organisms. Furthermore some of these micro-organisms may be visible in some stage of their growth or may be grown as has been done by Flexner and Noguchi with the organism of epidemic poliomyelitis.

Among the most important diseases due to filterable viruses are hog cholera, swamp fever of horses and sheep-pox found in domestic animals; yellow fever, dengue fever, poliomyelitis, scarlet fever and typhus fever in man; and rabies, foot and mouth disease, variola and vaccinia in man and some animals. In many of these diseases one attack confers immunity, for some an active artificial immunity has been induced but in few has it been possible to produce passive immunity. In some of these diseases certain species of bacteria have been quite uniformly found and there is a possibility that to produce these diseases the combined action of the filterable viruses and these bacteria is necessary. In hog cholera the demonstration of *Bac. suispestifer* and in scarlet fever the presence of streptococci certainly are of some diagnostic value.

VII. Leucocytes in the Circulating Blood in Infections.

For purposes of diagnosing infection both the number and kinds of white blood cells in the circulating blood are of importance. The normal blood contains 6,000 white blood cells per cubic millimeter, although the number may range from 4,000 to 10,000. Generally in infection with bacteria there is an in-

crease in the number of leucocytes in the circulating blood. The degree to which this increase occurs is regarded as an indicator of the degree of reaction of the individual to the infecting organisms. A high leucocyte count generally means a vigorous reaction while a low count means a poor reaction. The different species of bacteria differ in their ability to produce leucocytosis. Generally pneumococcus infection produces much leucocytosis; meningococci, staphylococci, streptococci and diphtheria bacilli produce some leucocytosis; in measles, malaria and tuberculosis there is usually no leucocytosis, and it is claimed by some that in typhoid fever there occurs an actual leucopenia. From this there are variations, the degree of leucocyte increase depending to some extent on the location of the infection, the efficiency with which it is localized and the degree of reaction. In various non-infectious diseases as splenomyelogenous leukemia the white cell count is increased while in other diseases it is decreased. This must always be taken into consideration when a diagnosis of infection is to be made from the leucocyte determination.

In addition to the pathological causes of leucocytosis there are certain physiological causes as ingestion of proteid-rich diet, pregnancy and so on. Malignant tumors, hæmorrhage, drugs and diseases of various nature may all increase the number of white blood cells in the circulating blood.

Recently greater stress has been given to the kinds of white blood cells in infections. It is unnecessary here to describe all of the types of white blood cells and to give the technique for making the determinations. Usually only the percentage of the different types of cells is determined but in some cases it is desirable to know their absolute number. In general it is fair to assume that an increase in polynuclear leucocytes speaks for bacterial infection while large numbers of mononuclear cells indicate protozoal infection. In infection with trichinæ, filaria, echinococci, tape worms and other animal parasites, and in certain types of neurasthenia the eosinophiles are increased. In syphilis and tuberculosis the mononuclear cells are proportionately increased. In well children the percentage of small mononuclear cells may be as high as 50 to 60 per cent. In some of

the non-infectious diseases, after hæmorrhage and so on, changes of great degree in the percentage of the different types of white blood cells are also observed.

Determination of the number and percentage of the different types of white blood cells in the circulating blood is of decided value in the diagnosis of infection. In most cases however determinations must be made at intervals, must be properly made, must be considered in relation to the symptoms and signs observed in the patient and above all must be properly interpreted. Blood examinations made at intervals of several hours showing increase in the number of leucocytes and especially of the polynuclear cells speak for pyogenic infection but absence of increase in white blood cells and polynuclear cells does not definitely exclude pyogenic infection. Any leucocyte count above 10,000 must be regarded as indicative of a pathological process. When the increase occurs within a few days or hours and if the polynuclear cells comprise 80 or more per cent. of all the white blood cells there in most cases is infection. By this means infection with streptococci, staphylococci, pneumococci, and so on can be diagnosed, abscesses can be differentiated from typhoid fever, tuberculosis and syphilis, perforation in typhoid can be diagnosed and so on.

CONCLUSIONS ON SPECIFIC DIAGNOSIS.—Gradually the laboratory tests have been regarded as more and more necessary to the making of a specific diagnosis. Physicians who at one time made and could make specific diagnosis are more frequently depending on laboratory methods and consultants. Some of this has undoubtedly been beneficial but it has also made it possible for many physicians to forget their other means of diagnosis. In all cases the demonstration of the etiological factor offers the best diagnosis but even this is not the only essential for some strains of disease-producing bacteria need not be disease-producing in all individuals. Micro-organisms not classed as pathogenic may because of putrefaction or fermentation produced by them be of great importance in the diagnosis and treatment of the disease in question. The laboratory consultants having the best success are beginning to insist on getting the history and learning the epidemiology and

the symptoms and signs of the disease to use in connection with their laboratory studies. From this clinicians should learn that results of laboratory tests only are to be regarded in the light of evidence on the cardinal symptoms of the disease.

CHAPTER IV.

SPECIFIC THERAPY.

In an earlier chapter the natural defensive forces of the body against infection have been discussed. It has been shown that the body has local powers of resistance and that even after they have fallen there still are forces naturally present to combat infection. For years the predisposing and depressing causes of disease have been recognized. When these have exerted their influence and the local and general barriers to disease have fallen, the body unless it is entirely overcome begins to elaborate certain other substances known as antibodies which combat the invading organisms. Even before the establishment of the germ theory of disease the production of especial protective substances was observed but since the development of our knowledge concerning immunity artificial attempts at the production and utilization of antibodies have become numerous. Specific antibodies to micro-organisms and their products now play a large part in specific diagnosis and therapy. Antibodies however are not the only substances of importance in specific therapy against diseases produced by micro-organisms as for many years certain chemical substances have been found to be specific in the treatment of certain diseases. Chemotherapy has been applied most successfully in the treatment of malaria and syphilis.

THERAPY DEPENDENT ON SPECIFIC ANTIBODIES.

The various forms of immunity have already been considered. Natural immunity is a non-susceptibility of species, race and family. In many cases it is only an apparent immunity and while it is inherited it cannot be transferred experimentally or artificially. Acquired immunity on the other hand is developed during the life of the individual or animal.

It is acquired either because the individual or animal has been subjected to a natural or modified course of the disease, or because the antibodies developed by another individual or animal are transferred to the one being immunized. The immunity developed by the individual is known as active immunity while the immunity he receives as the result of injection of antibodies that have been prepared for him is known as passive immunity. Both active and passive immunization are resorted to for curative and prophylactic purposes.

Prophylactic or protective immunization aims to supply sufficient specific antibody to prevent the development of disease. It is accomplished by active immunization and by the injection of antitoxic and antibacterial sera obtained from animals actively immunized. The duration of the protective influence acquired by the immunization varies but generally it may be said that the period of protection is shorter after passively acquired immunity than after actively acquired immunity. There are certain infectious diseases as smallpox against which we try to immunize all people while to other diseases protective immunization is only undertaken when there is an increased liability to infection. Thus the men in the army are immunized against typhoid fever and the children in families in which there is diphtheria receive protective doses of diphtheria antitoxin.

Curative immunization is undertaken when the infected body does not produce antibodies fast enough and in sufficient quantity to overcome the infection and when it is possible to introduce large amounts of specific antibodies which have been prepared in other animals. The effects of passive immunization if beneficial appear earlier than do those obtained on active immunization because in the immune sera the antibodies are already present while in active immunization they must be produced. For this at least several days are necessary.

The principles involved in immunization due to antibodies are well established even though the details of technique may vary in the different laboratories. Active immunization is essential in acquiring passive as well as active immunity so that in either case immunization means the successful produc-

tion of specific antibodies. To get proper antibodies in sufficient amounts the proper material (antigen) must be used to stimulate antibody formation and must be injected in proper amounts and at the proper time. The materials used for immunization have already been mentioned on page 9. Wright has shown that following injection of antigen there is a decrease of opsonin in the circulating blood. This he has called the "negative phase." It is followed by a positive phase in which the opsonin content increases for a time, later to decrease again in most cases. In order to get the greatest increase in opsonin Wright emphasizes the importance of injecting the proper dose at the time of the beginning of the decrease in opsonin content that is when the positive phase is beginning to subside. The doses and time between injections vary for different individuals and the various antigens. What holds true of the negative and positive phases, of antigen, its dose and time of injection as far as opsonins are concerned, undoubtedly also holds true as far as the indices of antitoxin, bacteriolysin and all the antibodies are concerned. The especial features will be considered in greater detail under vaccine therapy.

It is not to be assumed that antitoxins, bactericidins and opsonins are of equal importance in all infections. They are neither produced equally nor are they of equal value in overcoming the various infecting organisms. The antibodies of importance in immunization to infection should be established for the various pathogenic species. Meakins on this basis studied immunization to *Bact. dysenteriae*, *Streptococcus pyogenes*, *Micrococcus pyogenes*, and *Bact. tuberculosis*.

Wright and Douglas did not, as has been supposed by many, find that all pathogenic bacteria are sensible to opsonins. They divide the pathogenic bacteria into four groups:

(a) Those bacteria that are sensible to bactericidal, bacteriolytic and opsonic action of the normal human blood. *Bac. typhosus* and *Spir. cholerae* belong to this group.

(b) Those bacteria that are sensible to the opsonic and especially bactericidal action of normal blood serum. Examples of this group are to be found in *Bac. coli* and *Bact. dysenteriae*.

(c) Those bacteria that are definitely sensible to opsonin but not

to the bactericidal action of normal blood. *Bac. pestis*, *Mic. melitensis* and *Mic. pneumoniae* belong to this group.

(d) Those bacteria that are insensible to the opsonic and bactericidal action of normal human blood serum. Examples of this group are found in the bacillus of diphtheria.

The stimuli calling forth the production of antibodies vary markedly. The various antibodies of importance in the treatment and prevention of diseases are antitoxic or antibacterial. Antitoxin neutralizes toxin and agglutinin, precipitin, lysin and opsonin act on the bacteria. The value of these antibodies in immunization varies, agglutinins and precipitins being considered of little value except as evidences of the progress in antibody formation while bactericidal and antitoxic substances and opsonins are known to be factors in overcoming bacteria and their effects.

The production and injection of specific antibodies alone however is not sufficient for protective or curative purposes. It is necessary and essential that they be present in such parts of the body as to exert their influence where they are needed. In some infections the bacteria remain localized and at the point of localization may become so surrounded by tissue and other cells as to be protected from the antibodies which are not able to reach them because of increased viscosity of the blood, pressure on the blood and lymph vessels, stagnation, anatomical conditions, and so on. This condition is seen especially in furuncles, tubercles and in infections of the ventricles of the brain and canal of the cord. Various methods have been employed to supply antibodies to tissues and parts not easily reached during infection.

To decrease the viscosity of the blood citric acid in dram doses or sodium citrate in one-fourth to one dram doses given three times a day have proven of value in some cases. Both citric acid and sodium citrate have also been applied locally to decrease the viscosity of the blood and serum.

Evacuation of pus in abscesses is of value because usually the pus contains smaller amounts of antibody than does the serum, it furnishes a means for freeing the tissues of such bacteria as have been engulfed by the leucocytes, allows the more ready

access of serum containing antibody because of the release of pressure on the vessels and usually gives partial relief from pain. It is not to be assumed from this that incision of local infections is always to be practiced, the reasons for which are understood by experienced surgeons.

Hyperæmia to insure access of serum to infected areas is produced in various ways. Massage is of value in some cases. Heat in the form of poultices, hot water bottles, hot applications, dry heat to 200-300° F., electric light baths and Bier's method all have their special indications when it is essential to bring serum enriched in antibodies to the seat of infection. The value of hyperæmia however is not entirely due to the bringing of specific antibodies to the infected area. Langenbeck long ago made the observation that persons with stenosis at the pulmonary valves are likely to die of pulmonary tuberculosis while those with mitral regurgitation are relatively insusceptible to pulmonary tuberculosis. In pulmonary stenosis unless there is complete compensation there is a tendency to anemia and dry lungs while in mitral regurgitation the lungs are in a hyperæmic and moist condition. This suggests that venous hyperæmia and escape of fluid into the lungs opposes the growth of tubercle bacilli while a dry and anemic condition favors their development. This fact Bier has made use of in his venous or stasis hyperæmia which is accomplished by the use of suction cups and by the application of bandages in such a manner that the venous outflow is prevented but the arterial inflow is little or not hindered. While this is not the only value of the Bier method of treatment it is a very important one.

When anatomical conditions interfere with the ready access of antibodies as is the case in cerebrospinal meningitis and in tetanus, sera containing suitable antibodies are best injected directly into such parts. Injection of vaccines into infected areas is not practiced, in fact all indications are against such a procedure in active immunization. Local applications of antitoxic and antibacterial sera have been practiced with some degree of success. Attempts have also been made to apply to infected areas cultures and products of certain species of micro-

organisms for curative purposes. The value of these is still to be proven.

In all cases it must clearly be understood that if benefits are to be derived from antibodies these antibodies must have been produced in response to stimulation by the proper antigen. This makes it imperative to diagnose the species of organism causing the disease. Unfortunately many of the vaccines and some of the sera on the market are sold to the medical profession as vaccines or sera for boils, diphtheritic sore throat, and so on. Many of the preparations must be classed with "shot-gun" prescriptions. As long as physicians depend on the reading material in advertising circulars really valuable methods of treatment will be retarded in development, physicians will be discredited and patients deceived and defrauded. In no case should physicians employ remedies they do not know about especially when it is claimed that there is no necessity of making a definite diagnosis.

Many physicians and laymen have been deceived by what is referred to as "federal control of vaccines and sera." The various producers of viruses, sera, toxins and similar preparations use the label "Licensed by the Treasury Department," "U. S. Government License No. —," or "Guaranteed Under the Food and Drug Act." Of these the guarantee under the Food and Drug Act means nothing to the consumer—its only significance being that the manufacturer and not the dealer can be held. The license by the Treasury Department applies only to the products on the market in the District of Columbia or those entering interstate traffic. The license is necessary and is under the control of the Public Health and Marine Hospital Service. In the Hygienic Laboratory official American standards for diphtheria and tetanus antitoxins have been established and all such preparations sold in the United States must conform to these standards. Vaccine virus can be and is also well controlled but unfortunately there are a large number of vaccines and sera whose value has not been determined. In regard to these the best that can be done is to make certain that sanitary conditions exist in the stables, laboratory, and so on, and that the preparations are free from dangerous con-

tamination. Unfortunately manufacturers and dealers have used the "guarantee" as an evidence of the recommendation by the "Public Health Service," or have even said the "United States Government thinks enough of them to put its own guarantee on them." Physicians and the laity should bear in mind that for many products the Treasury Department license means nothing as far as the therapeutic value is concerned and that the guarantee under the Food and Drug Act is of no value to the consumer.

Vaccine Therapy.

For centuries it has been known that following attacks of certain acute infectious diseases there remains a certain loss of susceptibility to the contraction of a second attack of the same disease. Early in the eighteenth century this experience was utilized to produce immunity against smallpox. When it was found about the middle of the nineteenth century that many acute and infectious diseases have for their etiological factor certain micro-organisms known as bacteria, attempts were made to induce immunity by inoculation of killed or attenuated cultures of these organisms. Of the various attempts at active immunization by the injection of bacteria for prophylactic purposes, Pasteur's successful vaccination against chicken cholera is most important. Active immunization however against the various infectious diseases was not found to be feasible because it is not possible to immunize experimentally against all infectious diseases. The duration of immunity to some bacteria is short and there are so many disease-producing bacteria that the task of remaining immune to them all is out of the question. More recently active immunization for prophylactic purposes has been practiced except against smallpox only to protect the individual against such infectious diseases as he is likely to be exposed to. For this reason people living in cholera-infected districts are vaccinated against cholera, soldiers in the armies are immunized to the typhoid bacillus, and so on. Reports concerning the protection conferred by active prophylactic immunization differ. The mere presence of specific antibodies in the blood is not to be accepted as definite assur-

ance of the efficiency of vaccination and at present at least it is advisable for the physician to let the patient decide whether he wants to be actively immunized after the statistics and experimental evidence have been explained to him. Vaccination against smallpox affords the best evidence of the value of prophylactic immunization and nothing that has been stated here is to be interpreted as being opposed to compulsory vaccination against smallpox.

Active immunization to be applicable generally must be such that it is beneficial if induced after infection has occurred. Pasteur was successful in actively immunizing against hydrophobia if the immunization was begun before the symptoms and signs of the disease appeared. It was soon found that if active immunization was begun after the appearance of the disease there is a superimposition of a mild form of the disease on the severer form. This observation resulted in the disuse of the method until it was given a new impetus by Wright who depended upon the opsonic index as the guide to dosage and time of administration.

In an earlier chapter the different methods used in actively acquiring immunity have been given. The substances injected are either the bacteria, their products or their constituents and certain viruses. While the details of method and technique for active immunization vary, certain general principles are well established.

In active immunization the principle aim is to increase the amount of the antibody of importance in combating and overcoming the infecting organisms and their products. The various antibodies produced as a result of immunization have not all the same value in every infection and where two or more types of antibodies are produced in infection or immunization the amounts of each produced are not the same nor are they found to increase or decrease synchronously. It has been found in many tests that the agglutinating power of antiserum increases more rapidly than does the bactericidal power and that when the bactericidal power is at its height the agglutinating value may be low. In all cases therefore it is essential that the antibody of importance to a particular species of or-

ganisms be determined so that immunization can be regulated and the index for this antibody increased.

It is obvious that the antibody index may be affected in one of three ways:

(a) If injections are made after the index has returned to the normal the index curve may show a series of indices above and below the normal. This is what happens according to Wright when the individual is allowed to recover completely between injections. The curve is illustrated in Fig. 17A.

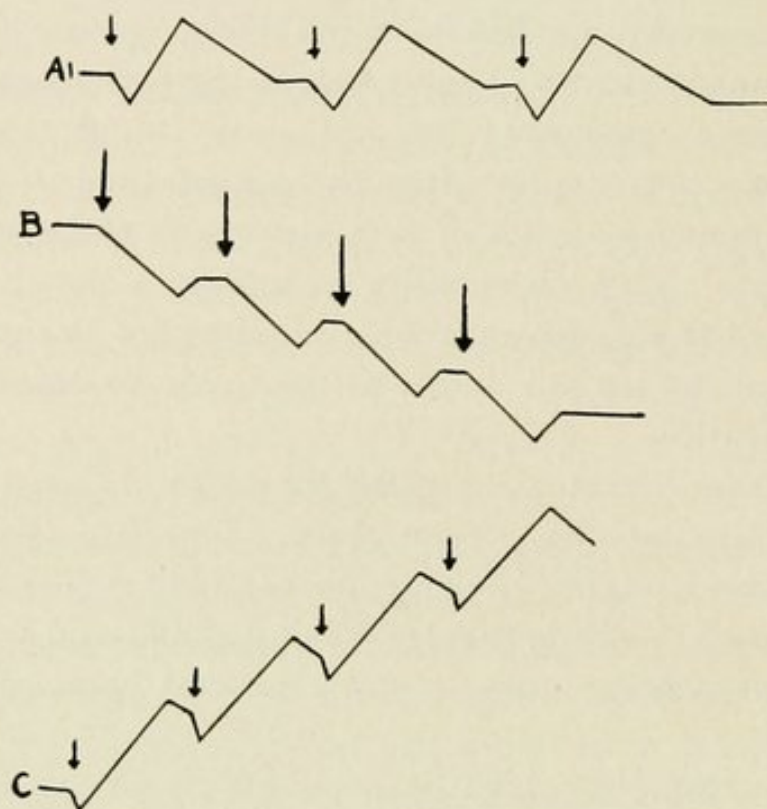


Fig. 17.—Schematic drawing to show how antibody index may be affected by injection of antigen.

(b) The injections may be given at such times that one negative phase is superimposed upon another negative phase and in this way produce a cumulative effect in the duration and degree of the negative phase. This result is obtained according to Wright when active immunization is pushed rapidly or forced. The curve of protective substance is shown in Fig. 17B.

(c) The injections may be made at such times and in such amounts that there is a summation of positive phases. This is

the result sought for in immunization. The curve representing the amount of protective substances in this case is represented in Fig. 17C.

The amount of antigen injected at a time and the interval between injections varies. It usually is not necessary to control the injections by determination of antibody indices. In most serum laboratories there is an established routine and in active immunization of patients to hydrophobia which has been highly successful, a routine is generally followed.

GENERAL CONSIDERATION OF BACTERIAL VACCINES.—Bacteriotherapy in acute infections was used by Beumer and Peiper, and Petruschky in the treatment of typhoid fever. Within recent years the application of bacteriotherapy for purposes of prophylactic immunization as well as for treatment of chronic and acute infections has received so much attention that especial consideration of bacterial vaccines is warranted.

Bacterial vaccines as generally understood are bacteria suspended in physiological salt solution, standardized to contain a definite number of bacteria in each cubic centimeter. Usually the bacteria are killed by heat while in suspension although living and attenuated organisms are used in some vaccines. The present day use of bacterial vaccines is based largely on the efforts of Wright and Douglas who advocate the production of active immunization by injections of such numbers of bacteria as will produce only a slight negative phase as determined by the opsonin content of the blood and followed by a rise in the opsonic index which lasts for some days. The second and subsequent injections according to Wright and Douglas should be made when the opsonic index begins to fall after the positive phase and at these times the injections should be such as to gradually produce an opsonic index above normal.

Selections of Bacteria for Preparing Bacterial Vaccines.—In all cases it must clearly be remembered that if benefits are to be derived from active immunization the vaccine must consist of a suspension of the causal organisms. To determine these microscopic examinations of the material from the lesion ought to be made and the organisms isolated when possible. This cannot be too strongly emphasized especially to the practi-

tioner who can buy from the druggist vaccines said to be good for boils, acne or whatever the condition may be while as a matter of fact the vaccine may not contain the organisms causing the particular lesion which is to be recovered from as a result of active immunization. This has undoubtedly been the cause of the failures observed by some clinicians and has, as was the case with tuberculin, decided them against the use of bacterial vaccines in certain cases of infection in which proper vaccines might have been of value.

Some investigators have used vaccines containing several species of bacteria. Serum and vaccine manufacturers have prepared vaccines suitable for active immunization against one or many different species. Wright at one time made no particular effort to identify the species of the causal organisms simply making a vaccine from the cultures obtained by inoculation with material from the lesion. This is open to many objections for at times the causal organisms may not grow on ordinary media or they may not grow as well as secondary invaders and saprophytic species. When mixed and undetermined vaccines are used in immunization it may frequently happen that those organisms for which immunization is necessary or beneficial are not injected. If mixed vaccines are to be used they should be prepared by mixing separate vaccines made from the different species.

Bacterial vaccines are of two kinds: the so-called "stock culture" vaccines and "personal" or "autogenous" vaccines. Before using stock vaccines it is necessary to determine the species causing infection while for autogenous vaccines it is only advisable to do so. Stock vaccines are to be made either from several different isolations of the species causing the infection or from a culture that has been found especially efficient in active immunization. Autogenous vaccines are made from the cultures of bacteria obtained from the patient. It has been claimed that for some organisms stock vaccines give as good results as autogenous vaccines and they have the advantage that no time need be lost in their preparation. Mixed vaccines consisting of the various species causing the infection and so-called "polyvalent" vaccines consisting of various strains

and species have also been used. When there are definite indications for using mixed and polyvalent vaccines they should be used but the use of mixed vaccines because of failure to diagnose the causal organisms is to be condemned. In all cases it is probably advisable to use autogenous vaccines when it is possible to do so.

Preparation of Bacterial Vaccines.—While killed cultures of bacteria had for some time been used in active immunization, the amounts injected had been only indefinitely determined. Wright and Douglas originated and described a method for the preparation and standardization of bacterial vaccines to be used in immunization. The causal organisms according to this method are grown on artificial culture media after which they are suspended in sterile salt solution. The number of bacteria per cubic centimeter of the suspension is determined by drawing into a capillary pipette (Figs. 9 and 10) one volume of the bacterial suspension and one or more volumes of fresh blood from a puncture in the finger. These volumes are then mixed well and a drop of the mixture placed on a clean slide. With a spreader which is placed in front of the drop a smear is made. This is then dried, fixed and stained with a suitable stain, usually Loeffler's methylene blue. On the slide the numbers of red blood cells and bacteria in from ten to twenty-five fields of the high power of the microscope are determined. Normal human blood contains five million red blood cells per cubic millimeter. After having established the ratio of red blood cells to the bacteria in the suspension, it is easy to determine the number of bacteria per cubic millimeter or cubic centimeter. The tube containing the bacterial suspension is now sealed and heated to 60° or 65° C. for one hour in a water bath. After killing the bacteria in the suspension, a dilution suitable for injection is made in a bottle containing 50 c. c. of sterile salt solution. The bottle is closed with a rubber cap and sufficient carbolic acid or lysol is added to make an ultimate dilution of 0.5 per cent. After this the vaccine is tested for sterility on suitable culture media. To obtain vaccine from the bottle a drop of pure lysol is placed on the rubber cap to sterilize its outer surface. Then the sterile hypodermic needle

is inserted through the cap, the bottle turned up and the piston of the syringe is withdrawn until the desired amount of vaccine has been taken into the barrel of the syringe.

Dosage.—Wright has by means of the opsonic index determined the proper dosage for the different bacterial vaccines and while he has not stated that definite amounts must be used has suggested certain doses. His principle is to use the smallest dose that will give a good rise in the opsonic index and never to increase the amount injected until it has been ascertained that the doses previously injected have been too small to produce a good rise in the opsonic index.

The doses of vaccine recommended by Wright are as follows:

<i>Bacillus coli</i>	5 to 50 million bacteria
<i>Micrococcus gonorrhoeæ</i>	5 to 50 million bacteria
<i>Micrococcus pneumoniae</i>	10 to 50 million bacteria
<i>Bacillus pyocyaneus</i>	5 to 5,000 million bacteria
<i>Micrococcus pyogenes</i>	50 to 1,000 million bacteria
<i>Streptococcus pyogenes</i>	10 to 25 million bacteria
<i>Bacillus typhosus</i>	5 to 50 million bacteria
Koch's New Tuberculin.....	1-1,000 to 1-400 milligrams

These doses are by some considered purely arbitrary because the personal characteristics of the individual to be immunized and the duration, extent and severity of the infection must all be considered in active immunization. While these doses may be arbitrary they at least offer some guide as to the number of organisms to be injected at a time. Active immunization to some bacteria may be accompanied by injection of specific immune serum. This may make larger doses and more powerful vaccine injections possible.

Injection of the doses recommended by Wright usually produces no constitutional symptoms. At times malaise, slight general muscular pain and headache are observed on the day following the injection but as the positive phase comes on there is a marked buoyancy of spirits and stimulation. Most investigators on this subject have never observed untoward symptoms when the small doses recommended by Wright are injected while others have at times observed a rise of temperature, nausea, vomiting, and so on. According to the principles

of active immunization probably the greatest amount of immunity results when there is some reaction.

The Site of Injection.—The site of injection of bacterial vaccines is considered by Wright to be of importance in the success of vaccination. Those regions of the body are selected where there is rapid absorption by the lymph and blood. It has been found that on subcutaneous injection greater amounts of immune body are formed than upon intravenous injection. This is especially illustrated in the production of certain hæmolytic sera. Leary has recommended the injection of vaccines into the muscular tissue because of the special immunity of these tissues, believing this to be evidence of their ready response to stimulation by bacterial vaccine.

While it is desirable to make injections as near to the point of infection as is possible and distal thereto, bacterial vaccines may be injected in any of the healthy subcutaneous cellular tissues. The regions preferred are the abdominal wall, the supra-scapular region, the gluteal muscles and the arm.

Control of Injections.—While Wright and his pupils have claimed to regulate the dosage and interspacing of injections entirely by the opsonic index, others have found the opsonic index too unreliable to serve as a guide in the administration of bacterial vaccines. Wright and his pupils have not always based their decisions on dosage and time for injection on determinations of the opsonic index for frequently at the time of the first injection of vaccine their patients have had an opsonic index well above normal. The time for the second and subsequent injection is not always controlled even by Wright and his pupils by the opsonic index.

Wright at various times has reported observations on the phagocytic reaction and opsonic index in infections running favorable and unfavorable courses. He has found that recovery and increased opsonic index accompany each other. Hektoen has reported that in pneumonia the opsonic index is at first low and rises as the patient's condition improves so that at the crisis the index is above normal. In patients that have a persistently low opsonic index in this disease death usually follows. Tunnicliff has found that in scarlet fever the opsonic

index for streptococci is below normal early in the disease and as the acute symptoms subside it rises above normal. Later on it again becomes normal. Ruediger has found in erysipelas a sharp rise in the index for streptococci as the temperature begins to fall. Hamilton isolated pseudo-diphtheria bacilli in 75 per cent. of cases of acute otitis media and found wide variation in the index for this organism in these cases. Clark has found that the opsonic index drops before a relapse in typhoid fever.

The writer in a study on the opsonic index in erysipelas made observations on the changes in the opsonic index in unvaccinated patients to determine the relation between the opsonic index for *Streptococcus erysipelatis* and recovery from, migration,

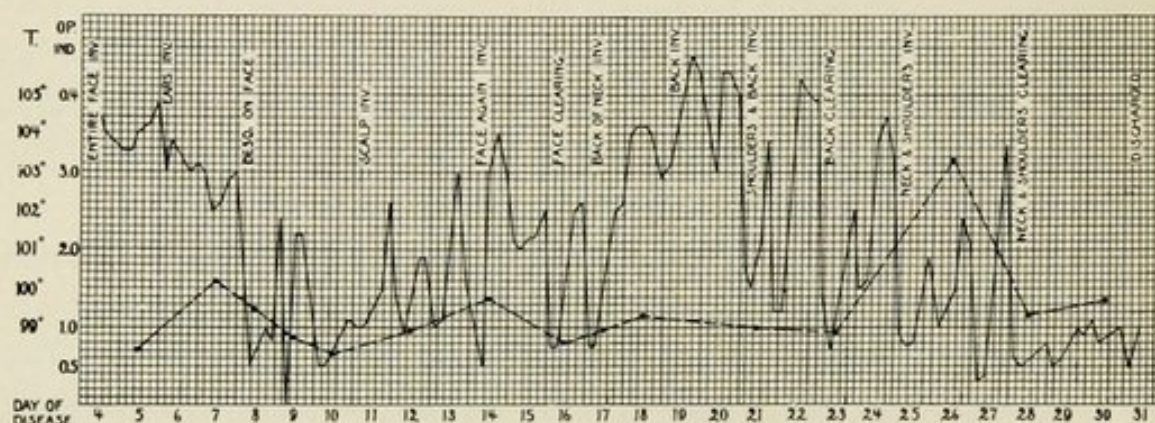


Chart I.—Temperature (unbroken line) and opsonic index (broken line) in a case of erysipelas (Case I).

recurrence and desquamation in erysipelas. The most instructive of these cases are the two which follow:

Case I. S., a man, aged thirty-eight years, who had a migratory, recurrent erysipelas of the face, ears, scalp and neck, was admitted to the hospital on the fourth day of his disease. When his face was involved his index was 0.7; when it was desquamating the index had risen to 1.3; when his face was again involved seven days later the index was 1.4; and when it was desquamating again, the index had dropped to 0.8. When his neck became involved one day after his face began to desquamate for the second time, his index was 1.0; two days later with an index of 1.1 his back became involved, and three days later with an index of 3.2 his shoulders and neck were again involved, and two days later with index of 1.1 the back and shoulders began to desquamate. (Chart I.)

Case II. A., a man, aged twenty-four years, suffering with erysipelas of the face and ears was admitted on the eighth day of the disease with an index of 0.9. The next day his temperature dropped and desquamation began; his index was found to be 1.1. Subsequently the index though the patient was perfectly well remained below unity. (Chart II.)

In these two cases there has been no constant change of index corresponding with desquamation and recovery.

To further study the relation of the opsonic index to the course of the disease, the indices of all patients who had received no vaccine were tabulated with regard to the day of the

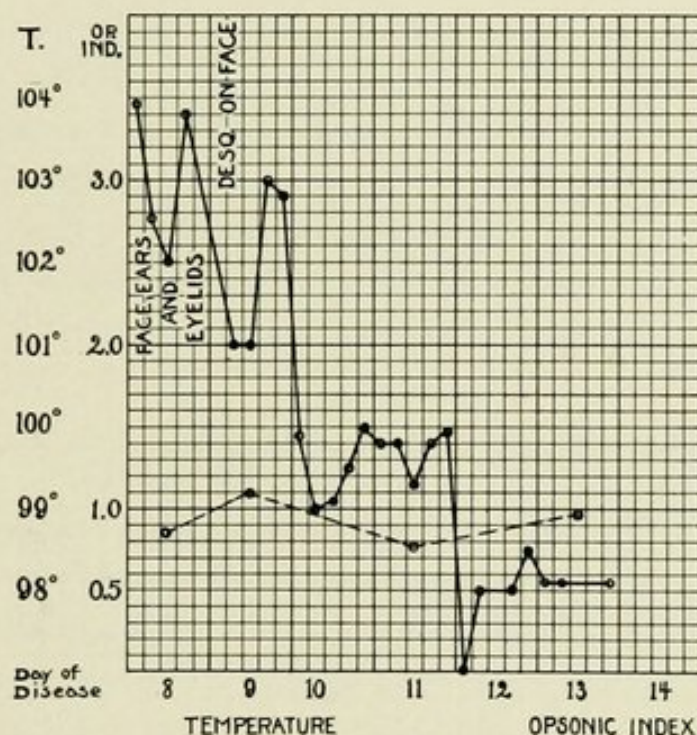


Chart II.—Temperature (unbroken line) and opsonic index (broken line) in a case of erysipelas (Case II).

disease and an average opsonic index for each day was determined.

TABLE SHOWING THE OPSONIC INDEX OF UNVACCINATED CASES OF Erysipelas on the various days of the disease.

Day of Disease.										
1	2	3	4	5	6	7	8	9	10	11
0.6	1.2	0.6	0.7	0.7	0.4	0.9	0.8	1.1	0.6	1.0
0.3	1.1	1.1	1.1	1.0		1.1	0.8	0.9		0.7
	1.0	2.4	1.4	0.9		0.6	1.3	1.7		1.0
		1.7	1.5	0.6		1.6				
			0.7	1.1						
			1.0							
			1.2							
0.45	1.1	1.45	1.1	0.86	0.4	1.05	0.96	1.23	0.6	0.9
Average.										

The table and composite chart (Chart III) indicate that erysipelas causes an increase of the opsonic index which reaches its maximum about the third day of the disease and is followed by a gradual fall. The subsequent course of the chart represents in a very large part observations made upon recurrent, migratory and complicated cases.

It is to be noted that the determinations made and represented in the last curve do not represent the index in any one case. The indices in individual cases are so variable and show such great irregularity that determinations of the opsonic index in any case give little indication of the severity of the disease and are of no value in prognosis. At present most investigators believe that in the natural course of infection there is no regu-

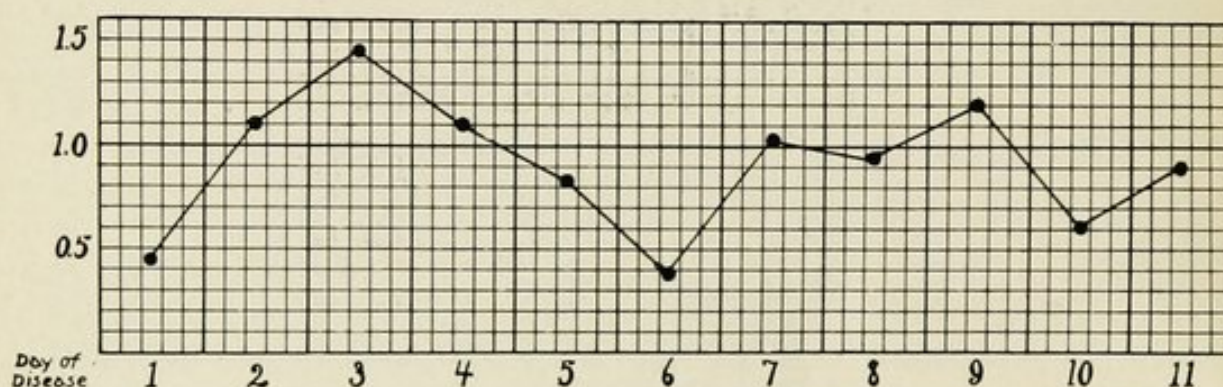


Chart III.—To show the average opsonic index and the day of disease when killed cultures of streptococci were not injected.

lar curve of the opsonic index and that in the natural recovery from an infection the changes in opsonic index are not those which Wright attempts to obtain by injection of killed cultures of bacteria.

While opsonins are undoubtedly of importance in immunity other antibodies must not be lost sight of. Various investigators have designed methods by which the action of agglutinins and bacteriolysins is to be inhibited so that opsonic indices may be determined and used as a guide for the dosage and interspacing of injections. The justifications for such regulation of dosage and determination of the proper time for injection are not evident. In a patient suffering with cerebrospinal meningitis from the spinal fluid of which *Mic. menin-*

gitidis was cultivated, the writer tried to govern the dosage and interspacing of injections of meningococcus vaccine by the opsonic index. After three to four minutes of incubation of the mixture of leucocytes, meningococcus suspension and patient's serum all of the organisms were found to be dissolved. It is evident that lysins in this case were probably of more importance in immunity than were opsonins. Clark has proposed that in determining the opsonic index of serum of typhoid patients the serum be heated to 56° C. in order to destroy the lysins for the typhoid bacillus. By following out this method Clark finds that relapses follow upon a drop in the opsonic index. How much more important would it probably be to determine the curve for lysins in a case where lysins are present in such amount that it is necessary to destroy their action before opsonic index determination can be made. According to the determinations of the opsonin content of the blood Wright and his pupils have found that the opsonic index begins to rise two to five days after injection and usually reaches its height after about five to eight days. In most cases adherents of the opsonin theory make injections every four to eight days. The appearance in the blood however of the better known antibodies occurs somewhat later. Bacteriolysins are found to be present at times in the spleen twenty-four hours after injection of culture. They do not however appear in quantities in the blood until five to nine or fourteen days later. Agglutinins are found to be present in the blood from eight to twelve days after injection of cultures. Typhoid agglutinins usually do not appear in a typhoid patient's blood until after ten days of the disease have elapsed. Furthermore it is to be noted that anti-toxins, bactericidins and agglutinins can be enormously increased by correct interspacing of injections and proper dosage. If injections are made entirely according to the opsonic index they may be made before the bactericidal and other protective substances have increased in amount and several negative phases be superimposed on each other, thus decreasing the protective substances. With the role of opsonins in immunity in doubt, the difference in sensibility of different species of micro-organisms to opsonins established and

the presence of marked bacteriolytic action of certain sera for certain species of infecting organisms observed, the control of the process of active immunization by the opsonic index alone does not seem justifiable. The immune bodies of importance in immunity to the particular species of bacteria ought to be established so that the dosage and interspacing of injections can be regulated by determinations of the indices of these substances. Such methods will undoubtedly result in a better accomplishment of a higher grade of active immunity.

Because of the shortcomings of opsonic index determinations and doubts in regard to their value clinical conditions have been much relied upon to determine the amount of bacterial vaccines injected and the interval between injections. Injections of bacterial vaccines are properly controlled clinically when there is improvement and recovery from the infection without or at most only slight local and constitutional symptoms. The general symptoms produced by injections of excessive doses are malaise, muscular pain, headache, nausea, vomiting, fever, and so on. The initial doses when controlled clinically are generally those recommended by Wright and the interval between injections is from five to ten days, which is the time required for the production of the known antibodies. In most clinics the doses injected are gradually increased. This is also the method recommended by Wright. It frequently happens however that following the larger injection there is an exacerbation in the pathological condition. The writer has obtained better results by injecting a large dose first and decreasing the doses in the second and subsequent injections. The reasons for following this method are based on clinical experience. The exacerbations following increased dosage may result from introducing so much vaccine as to fix the antibody and thus leave little to exert its influence on the viable organisms causing the infection or it may be due to an actual hypersensitivity to these micro-organisms.

ACTIVE IMMUNIZATION BY MEANS OF BACTERIAL CELL PROTEIDS AND BACTERIAL PRODUCTS.—The classical example of specific active immunization is the treatment of tuberculosis with the old tuberculin as first practiced by Koch. Attempts to produce

active immunization by the injection of bacterial cell plasma were made by Buchner who subjected bacteria to high pressure. Bacterial products have been used in immunization against glanders by injections of mallein. Bacterial toxins and products of bacterial autolysis have been injected to acquire active immunization and to produce antisera for passive immunization. In active immunization the value of the various bacterial cell constituents and products except the extracellular toxins is still doubtful. Rosenow's results on active immunization to pneumonia show that it is advantageous to remove the toxic material from pneumococci and only inject the part left after autolysis.

Physicians and investigators having before them the demonstration of the great value of vaccination against smallpox and the use of diphtheria antitoxin have tried to get equally efficient means for immunization against other infections. This has usually resulted in failure. In the interval between the publication of the favorable results of immunization by means of new bacterial extracts, antisera, and so on as reported by a discoverer and the establishment of the proof of the lack of their value, physicians have subjected a considerable number of patients to valueless and even dangerous methods of immunization.

Recently a preparation known under the trade name of "phylacogens" has been extensively advertised. Little literature concerning their use or preparation is available except that put out by the promoters. The originator holds that infections are usually due to a number of species of organisms and even though the diseased conditions are not all due to mixed infection the human subject is the host of so many species of micro-organisms capable of setting up a disease process that immunization against them all is necessary. For this reason he advocates mixed or "shotgun" injections for immunization. Phylacogens themselves are supposedly "sterile aqueous solutions of metabolic substances or derivatives generated by bacteria grown in artificial media"—whatever that may be. The injections are advised to be made preferably subcutaneously but may also be given intravenously. When given subcuta-

neously injections should be made only under the skin and not into the superficial fascia or muscle. Injections are to be repeated daily for five to six days. To be of value they should be followed by a local and general reaction with chill and fever. As has already been stated results are available only as published by the manufacturers. Opportunity has presented itself to me to see a number of patients treated by phylacogens and in no case could I see where beneficial results could be ascribed to the injection of phylacogens. Theoretically not much can be expected of them because bacterial extracts have been of little value in immunization. The toxic substances separable from some bacteria have been found not to call forth the production of immunizing substances but only to produce injurious effects. Active immunization of the human by the injection of intracellular toxins is relatively little practiced although extracellular or soluble toxins are injected in the immunization of animals designed to produce antitoxic sera to be used in passive immunization. Before crediting beneficial results to the use of phylacogens in pneumonia, rheumatism, and so on the physician must remember that most of the "cures" have occurred in self-limited diseases.

ACTIVE IMMUNIZATION TO DISEASES OF UNKNOWN ETIOLOGY.—Active immunization for prophylactic purposes was first accomplished for smallpox the etiology of which is still unknown. To-day vaccination against smallpox and immunization to rabies in which so-called "virus" is used in immunization stand out among the most effective measures in the prevention of communicable diseases. Active immunization to these diseases is obtained on injections of the virus located in some of the tissues of an infected animal. In the case of vaccination against smallpox the virus has been attenuated by passage through the calf or cow and in vaccination against rabies the virus in the spinal cord of rabbits with hydrophobia is attenuated by storage and dehydration. Similar methods have been tried in immunization of animals to other diseases of unknown etiology but of all vaccination against smallpox and hydrophobia stand out most prominently. Due to the long incubation period in hydrophobia active immunization here is

unique in that it need not be undertaken until after infection has occurred, while to most diseases immunization for prophylactic purposes must be begun before infection takes place.

Administration of Vaccine by Methods Other Than Injection.—Various methods have been suggested. It was noted by Meakins, Wheeler, Wright and others that when some tuberculous patients exercise there is an increase in the opsonic index. This has been supposed to be due to autoinoculation by absorption of minute doses of toxic substances forced out by muscular movements. Attempts have been made to produce this same result by massage and exercise in other infections. Vaccines have been administered by the mouth and in some cases by the rectum. The value of these various methods of administration has not been clearly established but usually they have been found to be unsatisfactory.

Serum Therapy.

In the discussion of theories of immunity mention was made of the fact that in 1887 Fodor showed that the fluids of the normal living body especially the blood possess bacteria-destroying properties. Buchner, Behring and Nuttall were among the first to corroborate the results of Fodor. It was later recognized that the cell-free blood serum of normal animals possesses properties of destroying bacteria. It is to be noted however that the blood of every species of animal will not destroy all species of bacteria. Furthermore while in certain cases the degree of natural immunity corresponds to the amount of the bacteria-destroying substance present still these two conditions are not always found in the same case. The substance which destroys bacteria Buchner called "alexine."

In 1888 Hericourt and Richet made the observation that blood serum from an animal immunized to *Staphylococcus pyosepticus* when injected intraperitoneally into rabbits confers an immunity to this organism. Babes and Lepp in 1889 reported the possibility of protecting animals against rabies by the injection of body fluid from animals immunized to rabies. These results were given but little attention until Behring and his pupils systematically investigated the subject. Behring and

Kitasato in 1890 reported that mice can be immunized to tetanus by the injection of blood serum obtained from rabbits artificially immunized. These investigators further reported that serum from tetanus-immune animals protects against the primary intoxication produced by the tetanus bacillus. In 1888 Roux and Yersin discovered diphtheria toxin. Behring and Wernicke in 1891 showed that blood serum from diphtheria-immune guinea-pigs or rabbits when injected intraperitoneally protects against and cures diphtheria. In 1892 these investigators further found that it is possible to immunize larger animals to diphtheria and demonstrated in the blood of these animals protective and curative substances which can be transferred in the serum to other animals. The doses necessary to cure the disease were found to be larger than those required to immunize against the same. The amount of protective and curative substance produced was found to vary to some extent at least with the degree of active immunization of the animal from which the serum was obtained. It was further found by Behring that serum from animals immunized to diphtheria will protect against the toxin of Roux and Yersin.

Based on these observations Behring formulated a law which is generally known as "Behring's Law." According to this dictum injection of blood or blood serum from an animal possessing acquired immunity confers immunity to susceptible animals or individuals if the right amounts are injected. Injection of blood or blood serum from an animal or individual having natural immunity does not confer this immunity to animals or individuals naturally susceptible. The immune substances that can be transmitted with the blood or blood serum are not present naturally but must be produced by a process of immunization consisting either of having the disease or submitting to some method of artificial immunization. Conference of immunity by the injection of serum from animals having acquired an immunity is known as passive immunization. The development of methods and success obtained by passive immunization against tetanus and diphtheria bacilli led to the belief that it would be a relatively easy matter to make efficient specific sera against all disease-producing bacteria. It was soon

found that this is not possible and that antisera differ in their value as prophylactic and curative agents.

In an earlier chapter the toxins produced by pathogenic bacteria have been classified into extracellular and intracellular. Conditions favorable to the liberation of toxins by bacteria grown in artificial media have been produced for only a few bacterial species, most of the species either not liberating their special poison until the organisms disintegrate or else producing their poisons only under the conditions that prevail in the susceptible body. This has necessarily limited experimental immunization to the toxins of only a few bacterial species, experimental immunization to all other pathogenic species being acquired by the injection of bacteria, their products, autolysates, and so on. Immunity to toxins is known as antitoxic immunity, that to bacteria as antibacterial immunity and the sera from animals actively immunized by injections of extracellular toxins are known as antitoxic sera, while sera from animals actively immunized to bacteria, their plasma and so on are known as antibacterial sera.

It was stated earlier that sera containing bodies which neutralize toxin are called *antitoxic sera*. The immune bodies are receptors of the first order of Ehrlich (see p. 17), thrown off as a result of overproduction of certain receptors. Overproduction of these receptors occurs as a result of the presence of the specific toxins produced by certain organisms or after experimental injections of certain toxins. As is the case with other antibodies antitoxins are present to some degree in many normal individuals. Thus diphtheria antitoxin was found by Bolton to be present in the blood serum of 30 per cent. of the horses examined and in 50 per cent. of the children and 81 per cent. of the adults examined by Wassermann. A considerable number of antitoxic sera have been made, the principal ones being for the toxins produced in infections with the organisms causing diphtheria, tetanus, symptomatic anthrax, as well as for the toxins produced by *Bac. pyocyaneus* and *Bact. botulinus*. Antitoxic sera have also been used to combat intoxication caused by snake and spider bites and by poisoning with toadstools and poison ivy. Antitoxins have been produced for other toxins as ricin, abrin

and robin but these have been little considered as compared with the antitoxins in antidiphtheritic and antitetanic sera.

Sera containing substances which result in the death and destruction of bacteria are called *antibacterial sera*. Acquired antibacterial immunity depends on destruction of bacteria before they have had chance to multiply and produce sufficient amounts of poison to kill the cells in the body. For this reason antibacterial sera are frequently called bactericidal sera. In the serum of individuals and animals that have acquired antibacterial immunity various kinds of antibodies are found. The best known of these bodies are bacteriolysins, opsonins, antiendotoxins, precipitins and agglutinins. These prepared antibodies are introduced into the body in passive immunization. The importance and value in immunity of all of these antibodies is not known but it is generally accepted that agglutinins and precipitins are of little or no value while opsonins, receptors of the third order causing complement fixation and bacteriolysins are considered of importance in antibacterial immunity. Bacteriolytic immune sera depend for their action on specific substances called "bacteriolysins" which dissolve bacteria. The value of opsonins in bactericidal sera to be used in injections for the purpose of conferring passive immunity is still indefinitely understood although some investigators have attached considerable importance to their presence in sera.

The antibodies called agglutinins and precipitins belong to the second order of receptors, the bacteriolysins to the third order while in regard to opsonins no statement can be made at the present time—some regarding them as belonging to the second and others to the third order of receptors. Antisera containing receptors of either the second or third order are easily inactivated by heat, age, acids and so on. After inactivation they are no longer able to produce agglutination, precipitation, lysis or opsonification. The loss of power to produce these effects depends on the destruction of ferment-like substances which are a part of the agglutinin and precipitin receptors and are furnished to the receptors of the third order by the fresh blood serum (see p. 20). Inactivated bacteriolytic serum differs from inactivated or aged agglutinating

or precipitating serum in that on the addition of fresh serum the antibodies producing lysis can be re-activated while the agglutinating and precipitating properties cannot be restored in this way. Because the different specific bacteriolytic sera used in passive immunization are usually not freshly drawn and therefore have been inactivated by age, the individual or animal immunized must furnish the complement or ferment-like substance so as to make the destruction of bacteria possible. The normal individual in order to utilize all the lytic and possibly also the opsonic antibodies in a specific serum therefore must possess large amounts of complement. Numerous investigators among whom may be mentioned Ehrlich, Morgenroth and Metchnikoff have found that in certain diseased conditions there is an actual decrease in complement. As far as can be determined complement is not increased during disease and immunization. Richardson has shown that usually the serum of typhoid patients is not able to destroy typhoid bacilli but that upon addition of fresh serum from a normal individual the typhoid immune serum is able to bring about complete lysis. Numerous similar observations have been made in natural infections in man. In the efficiency of antibacterial sera the presence of sufficient amounts of complement undoubtedly plays a large part and in certain cases the lack of complement probably is the cause of failure of beneficial action of specific antibacterial serum. Various attempts have been made to increase the amount of complement. Thus anticomplement has been used to immunize in order to excite the over-production of complement. Injection of fresh complement with the immune serum has been tried by some investigators. Efforts have also been made to preserve the complement by freezing the serum and keeping it cold until it is injected. Dried immune sera have been advocated because it is known that complement is preserved for a long time in serum dried down soon after it is drawn. It is questionable however whether by any of these methods enough complement will be present to activate the amount of immune bodies injected. Hiss has published results on the curative action of subcutaneous and intraperitoneal injection of extracts of leucocytes from normal rabbits.

Of this leucocytic extract he says: "The action of the leucocytic extract may be due to the enhancement of the bacteriolytic action of the animal's plasma by the introduction of complement—but is most likely chiefly due to the poison-neutralizing or destroying bodies."

The practical application of specific antibacterial sera in the treatment of the various infections in man has been tried on a large scale. It has been found that by the early injection of serum from animals actively immunized other animals can be protected against infections with pneumococci, gonococci, streptococci, typhoid and dysentery bacilli, cholera spirillæ and many other micro-organisms. Based on these results, many different antibacterial sera have been used in passive immunization in man, the most important of these being the sera which contain specific substances by which they destroy streptococci, meningococci, pneumococci, typhoid and dysentery bacilli, and staphylococci. The results of antibacterial sera in the treatment of diseases of man have however not been as satisfactory as those obtained with specific antitoxic sera.

Standardization of Immune Sera.—In the use of all curative or remedial agents it is highly important that a dose may be established and in order that this may be done it is necessary that the strength or potency of the agent be known. This holds true for the various antisera and consequently attempts have been made to standardize them. As has been stated before the antisera belong to one of two classes, antitoxic or antibacterial. It has been found that it is relatively easy to determine the value of antitoxic sera but for the antibacterial sera at present no satisfactory methods have been worked out.

The methods for standardizing antitoxic sera have varied some but now in the United States fairly uniform and satisfactory methods are in use for the standardization of the two principal antitoxic sera, antidiphtheritic and antitetanic. A standard test toxin is provided by the Hygienic Laboratory of the United States Public Health Service with which the toxin of the local laboratory is standardized. After this has been done the smallest dose fatal to a susceptible animal of given weight is determined and then the smallest amount of

serum containing enough antitoxin to protect against a multiple (for diphtheria 100 times and tetanus 1,000 times) of this smallest fatal dose of toxin is ascertained. This amount of serum contains one antitoxic unit.

The methods for standardizing antibacterial sera are less satisfactory. Mention has already been made of the fact that antibacterial sera are of value because of one or more of the different classes of antibodies. Likewise it has been stated that of these antibodies agglutinins and precipitins are probably of no value in immunization but that bacteriolysins, opsonins and anti-endotoxins are. Before attempts at standardization of antibacterial sera are made it seems essential to determine which of the antibodies are of importance in combating the bacterial infection and the smallest fatal dose of the various species of micro-organisms. Attempts to determine the minimal fatal dose for different species have been made but it must be remembered that with living micro-organisms a multiple of the minimal dose is not as potent as the multiple would indicate. For this reason standardization based on the injection of multiples of the minimal fatal number of bacteria followed by injections of antiserum or the simultaneous injection of bacteria and antiserum has been of little value. Probably the best methods of determining to some extent at least the potency of antibacterial sera is from the bacteriolytic and opsonifying value of the same. For some of the antibacterial sera the high agglutinating value is claimed as an evidence of potency. It has been mentioned repeatedly that agglutinins are of little or no protection. Furthermore the agglutinating power need not necessarily be an index of the bacteriolytic and opsonic indices. It may be accepted that generally for antibacterial sera there is no reliable standardization and that the dosage is determined from the cubic centimeters necessary to produce beneficial results.

Concentration and Purification of Serum.—Frequently large amounts of specific immune substances must be injected in the treatment of infections. Hypodermic injection of large amounts of serum however is painful and more likely to produce serum disease especially the rashes. These objectionable

features of serum therapy have resulted in an attempt to produce serum of greater potency.

It has been found that bacteria and toxins from certain cultures are more potent and cause animals to produce immune sera of higher protective value. As a result virulent cultures or toxins produced by virulent cultures have been used in active immunization of the animal that is to furnish the immune serum.

Attempts have been made to obtain an increased potency by separating the immune substance from the non-immune substance of specific serum. Dieudonné in 1897 showed that the proteids precipitated from diphtheria antitoxin by acetic and carbonic acids contain most of the antitoxins. In this same year Belfanti and Carbone found that antitoxins are precipitated with the globulins of antitoxic sera by magnesium sulphate. Atkinson in 1901 and others since then have shown that during immunization the serum-globulin content of serum increases. Gibson and Banzhaf found that this increase in serum-globulin is not necessarily associated with the accumulation of antitoxin in the blood. Early in 1906 Gibson working in the Research Laboratories of the Department of Health of New York City reported practical methods for concentrating and purifying diphtheria antitoxins, his especial contribution being based on the observation that the globulins, nucleoproteids and so on carrying the antitoxin when precipitated with magnesium sulphate are again soluble in a saturated sodium chloride solution. According to Gibson's method by half saturation of antitoxic serum with ammonium sulphate the globulins, nucleoproteids and similar substances are thrown out. The precipitate is then again dissolved in a saturated solution of sodium chloride. Now only the antitoxic fraction and some of the globulins are in solution. After filtration acetic acid is added to the filtrate to precipitate the antitoxic globulins. These are then filtered off, dried with paper, dialyzed, neutralized and again dialyzed for several days. After dialysis the solution is made isotonic by the addition of sodium chloride. The solution is then filtered through a Berkefeld filter to remove any bacteria that may be present and chloro-

form or some other substance added as an antiseptic. This method has been attempted for the various immune sera but up to the present time it is profitable only for the concentration of diphtheria and tetanus antitoxins. Later Banzhaf found that more concentrated antitoxin may be obtained by heating the serum to 57° C. for 18 hours before separating the antitoxic globulins for the antitoxic fraction remains in solution while the globulins not antitoxic precipitate out. By Gibson's method the concentration is increased from three to five times while by Banzhaf's from eight to ten times.

By the use of concentrated and refined serum the constitutional disturbances and rashes are somewhat less frequent and not as severe. The principal advantage of the product obtained by these methods however lies in the fact that a large amount of antibody can be injected with small amounts of material.

Relatively recently immune sera especially antidiphtheritic and antitetanic have been dried after concentration and refinement. It was mentioned earlier that when serum is dried soon after being drawn the immune body and especially the complement are preserved longer. Among the advantages of dried immune sera may be mentioned: the lack of deterioration, the cheapness of manufacture, the convenience of carrying about in the medicine case and the possible administration of immune substances by the mouth. Possibly dried sera will prove to be of considerable value and are not to be lost sight of.

Indications for the Injection of Antisera.—Sera containing specific antibodies are used for prophylactic and curative purposes. The indications for the injection of antisera for prophylactic purposes vary a good deal. Some physicians use the sera indiscriminately, in some hospitals all children admitted as patients receive immunizing doses of diphtheria antitoxin, other physicians inject all persons exposed with immunizing doses of specific antisera, and so on. Gradually the indiscriminate use of sera has been decreased. The especial indications for the use of the different antisera are given in the succeeding chapter and here only the essential points are referred to. Immunity conferred by the injection of antisera is of relatively short

duration so that if the individual is to remain immune the injections must be repeated after each exposure to the disease. This may lead to such undesirable results as serum sickness, development of antibodies to the antibodies of the serum and the deflection of complement. In all cases where serum injections for prophylactic purposes are contemplated it is important to remember the foregoing facts. If the curative value of a serum is as great as that of diphtheria antitoxin, prophylactic injections are seldom indicated.

Injections of specific antisera for curative purposes should be based on the diagnosis of the etiological factor. To this there are but few exceptions. In certain cases the clinician is warranted in using a specific serum on the basis of clinical evidence or when the patient has been exposed to infection by a previously diagnosed case of the communicable disease. When it has been decided that a specific antiserum is to be used two important principles must be remembered, first early administration and second injection of sufficient amounts to produce therapeutic effects.

In the treatment of diseases for which antitoxic sera have been made it must always be remembered that antitoxin neutralizes prepared toxin and combination can take place only when the receptors of the toxins and antitoxins are free. As soon as the cells and toxins have combined and the body cells have been injured as a consequence no amount of antitoxin can protect the cell from the action of the poison molecule. Antitoxic bodies can only anchor and render inert or harmless such toxins as are free or have uncombined receptors. This emphasizes the importance of the early administration of antitoxic serum because at this time the poison molecules can be anchored by the antitoxic substances instead of combining with the cells of the body. Antibacterial substances with the aid of complement have the power of destroying bacteria but not of neutralizing bacterial toxins. For this reason it is essential that antibacterial sera be used so early in the disease that the bacteria have not had a chance to multiply or set free enough poison to injure and kill the body cells. Early injection of specific sera unfortunately is not possible in many of the dis-

eases for which specific antibacterial sera have been used but as our methods of early diagnosis are improved earlier administration of these sera can be made. It is to be remembered that it is of the utmost importance that these sera be used before the infecting organisms have multiplied greatly. Another reason for the failure of antibacterial sera is dependent upon the fact that as the bacteria are dissolved and destroyed by the action of immune substance and complement the intracellular toxins are liberated. If the body cells are not able to cope with the increased amount of toxin liberated upon the solution of the bacteria some of the body cells or even the organism may die. Hiss has produced evidence to show that the leucocytes contain substances neutralizing these poisons. To have liberated the minimum amount of toxin antibacterial sera should be administered when the number of invading bacteria is relatively small, that is early in the disease.

The amount of serum injected varies but in every case therapeutic effects should be noted several hours after injection. If enough has not been injected at first another dose should be injected several hours later. The dosages for antitoxic sera are better established than for the antibacterial sera. Antisera of various doses are furnished in syringes by the different supply houses so that it is relatively easy to inject any amount desired.

Administration of Antiserum.—Serum is usually administered into the subcutaneous tissue, the injections being made in any part of the body where the skin is loose. The sites most frequently selected are between the scapulæ, on the abdomen or on the breasts. Before the injection is made the skin at the site of injection should be well cleaned with soap and water, alcohol and some antiseptic solution as 5 per cent. solution of carbolic acid or the tincture of iodine. The entire operation should be done with aseptic precautions. Before inserting the needle the air should be removed from the syringe and the plunger pushed in a short distance to be certain that the syringe will work properly. After the needle has been inserted the serum should be forced in slowly to give the least pain. It is advisable not to massage the injected tissues.

Some sera, as the serum used in the treatment of epidemic cerebrospinal meningitis, are injected into the spinal canal. Sera are at times given intravenously. The technique for this is much like that for obtaining blood for blood cultures except that the tourniquet is removed as soon as the vein has been entered. Sera have also been applied locally: for example, diphtheria antitoxin has been applied directly to the membrane.

The possibility of oral administration of antitoxic sera has been advocated by McClintock and King. In 1892 Ehrlich discovered that ricin antitoxin can be absorbed by the intestinal canal. In 1893 Wernicke working with Behring established the fact that antitoxic substances in the body fluids of diphtheria-immune animals are absorbed by the digestive tract; thus dogs fed on the meat of diphtheria-immune sheep obtain some immunity to diphtheria. McClintock and King have found that the best results by oral administration of diphtheria antitoxin are obtained by the following method: "One-half hour before administering the serum the child is given one glass of one per cent. sodium bicarbonate solution. When the antitoxin is given there is added one minim Fl. Ext. Opii and from four to ten minims of saturated solution of salol in chloroform. When possible no food should be given for at least four hours before administering the serum." This procedure is adopted to inhibit the digestion of the antitoxin so as to make the absorption of the unchanged antitoxin possible. Dried diphtheria antitoxic globulins were found to give satisfactory results. By oral administration of diphtheria antitoxin neither serum sickness nor anaphylaxis was observed. At present this method is still of questionable value and requires further investigation.

Untoward Effects of Serum Injections.—Injections of antisera are at times followed by untoward or undesirable effects. These may be due to various causes among which may be mentioned faults of the serum, development of undesirable antibodies, foreign proteids and so on. The sera to be injected in all cases should be sterile and remain so and usually although not always the sera on the market have this virtue. Sera may also be faulty because not all of the toxin or culture injected

during immunization of the animal is fixed so that toxins or bacteria remain in the serum. Bacteria from these sera are usually removed by filtration but the free toxin may lead to added intoxication of the patient. Repeated injection of antisera may result in the production of anti-antibody. Some sera are only of value if there is sufficient complement. If complement in fresh serum is injected anticomplement is formed in most cases. Anti-antibody and anticomplement formation are of relatively little importance in our present methods of the use of antisera.

In the conference of passive immunity blood and blood serum injections are made almost exclusively. Blood transfusions were probably first made by Denis. While some good results were obtained fever, emboli, bleeding, hæmoglobinuria and urticaria were sometimes produced by these blood transfusions. Until 1894 when diphtheria antitoxin was first generally used injections of blood and blood serum were rather rare. Following the injection of blood from animals immunized to diphtheria toxin exanthemata were observed in about 22 per cent. of the cases, while the injection of only the blood serum produced exanthemata in 6 or 7 per cent. of the patients. From time to time skin eruptions and other untoward effects of serum injections have been reported. These sequellæ have usually not been serious still at times serious symptoms and even death have been reported as results of serum injections.

In 1905 von Pirquet and Schick originated the term "Serum Krankheit," or serum disease. These investigators found that serum disease varies, two rather definite types of the disease being recognized: one type which follows the first injection of serum and another type which follows the second and subsequent injections.

Serum disease following the first injection of serum manifests itself after an incubation period of eight to twelve days and is largely independent of the amount of serum injected. Of the symptoms of this disease fever is the most constant, lasting usually during the entire course of the disease. The height of fever however is not an indicator of the disease but from the curve of the temperature a prognosis can be made because the

temperature drops by lysis. Together with fever there is usually an exanthema appearing most frequently as urticaria. This appears first about the point of injection. Later it is distributed symmetrically and usually itches severely. At times there is swelling of the glands especially of those found in the region injected. The symptoms of pain in the glands are of prognostic value inasmuch as the pain usually disappears or abates before the size of the gland decreases. During the period of incubation the leucocytes are increased in number but during the height of the disease the number of leucocytes is markedly diminished. Joint pains are found in a small percentage of the cases of serum disease. The metacarpal, hand and knee joints are most frequently affected. Usually these pains do not last long. In certain cases there is edema but albuminuria is seldom or never present. The mucous membrane is seldom affected and thus distinguishes the condition from scarlet fever and measles. The disease is further distinguished from measles by the absence of Koplick spots, coryza, conjunctivitis and by increased efflorescence about the point of injection. From scarlet fever the condition is distinguished by its noncommunicability by contact and absence of scaling, nephritis and angina.

The disease following the second and subsequent injections of serum according to von Pirquet and Schick varies somewhat with the interval between injections. If an injection of serum is made from twelve to forty days after a preceding one the incubation period is very short, the disease appearing at times in less than one hour after injection. If the interval between injections is from forty days to six months there may be an immediate reaction or else an immediate reaction with another reaction six to eight days later. If the interval between injections is over six months there is usually no immediate reaction, the symptoms appearing six to twelve days after injection. When the interval between injections is six days or less the disease is not produced. Serum disease follows subsequent injections more frequently than it does first injections and is usually produced by smaller amounts of serum. The "immediate reaction" occurring when re-injection is made within an

interval of twelve to forty days manifests itself in one to six hours after injection and usually reaches its maximum within twenty-four hours after injection. The "second reaction" does not occur after the first injection of serum but occurs most frequently when the interval between injections is between forty days and six months. The incubation period for the second reaction is somewhat shorter than for serum disease produced by first injection. The symptoms of serum disease produced by subsequent injection are usually more acute and more general but of shorter duration. In many cases there is vomiting.

When serum disease was first recognized it was supposed to be caused by toxin in the immune serum. However as early as 1894 Heubner expressed doubt as to the importance of specific immune sera in the production of the disease. Later the manifestations of serum disease were produced by the injection of normal serum. In 1906 Rosenau and Anderson of the Hygienic Laboratory of the Public Health and Marine Hospital Service of the United States published a work in which experiments were reported on "sudden death" of guinea-pigs following serum injections. These investigators found that injections of normal horse serum into guinea-pigs are very poisonous if the interval between injections is more than ten days. The length of time for which this hypersusceptibility persists has not been definitely determined but lasts at least as long as 1096 days (little over three years)¹. On the other hand when the interval between injections is less than ten days normal horse serum will produce no such effect.

The nature and causes of the reaction have been matters of considerable investigation and contention. The reaction was at first regarded as one resulting from the injection of toxin or poison. The long period of incubation however is explained with difficulty by such a conception of the phenomenon. While it is true that to produce disease by certain toxins a period of incubation is necessary, this period is seldom as long as eight to twelve days. It can hardly be conceived that horse serum has the ability to increase its amount of poison as can bacteria and other organisms capable of self-reproduction. Rosenau and

¹Hygienic Laboratory. Bull. No. 50, Apr., 1909.

Anderson have determined quite definitely that the reaction is due to a difference in the susceptibility of individuals and not the toxicity of the serum.

Von Pirquet and Schick conclude that the phenomenon is due to the presence of specific antibodies. According to these investigators the antibodies are not precipitins. Man does not produce the precipitins readily with horse serum, in children three weeks must elapse before the precipitins can be detected and they persist in the blood only from four to nine weeks. The "immediate reaction" which occurs when the second injection of horse serum is made twelve to forty days after the first, von Pirquet and Schick explain as being due to the serum antibodies already present which combine with the immunizing substance in serum and produce poisonous substances. These in turn produce the disease. According to these investigators the reaction is one between antibody and antigen.

Gay and Southard in 1907 reported experiments which indicate that the theory of von Pirquet and Schick is untenable. These investigators believe that "sudden death" in guinea-pigs "sensitized to horse serum" is due to a substance they call "anaphylactine." This substance is found in normal horse serum, is not absorbed by the tissues of the guinea-pig and is eliminated slowly. The anaphylactine in the guinea-pig increases the avidity in the cells of the guinea-pig so that when more serum is injected the cells are "overwhelmed in the exercise of their eliminating functions and functional equilibrium is so disturbed that local or general death may follow." Rosenau and Anderson had shown that the hypersusceptibility of the guinea-pig for a certain proteid is manifested upon a second injection of the corresponding proteid. They showed that the substance anaphylactine is specific in the same sense. These investigators could not demonstrate this substance in the blood of the sensitized guinea-pig during the incubation period nor at any time in the blood serum of man, monkeys and cats. Later Rosenau and Anderson presented evidence that antibodies are concerned in the phenomenon of anaphylaxis. Weil¹ believes that an animal that is anaphylactic does not have

¹Weil: J. Med. Research, 1913, XXVII, 497.

enough antibody to protect the body cells and that the antibody in immunity and anaphylaxis is the same but in immunity it is present in sufficient amount in the serum to protect the cells while in anaphylaxis the serum does not contain sufficient antibody to prevent the antigen from combining with the antibody anchored to the body cells.

It is now quite generally accepted that specific antibodies called "Allergins" are responsible for anaphylaxis and that anaphylaxis may be induced passively in animals by injection of serum from sensitized animals. During the stage of anaphylactic shock when there are especially symptoms of asphyxiation there are not enough free allergins to confer passive sensitization. The structure of the anaphylactic body is unknown but apparently the non-specific complement is necessary. The action of the antibody is generally regarded as being quantitative.

Sensitization occurs only to foreign bodies. When proteids are taken into the system usually they are split up and prepared for assimilation by the body tissues. Now while the body cells may have receptor apparatus for these derivatives of proteids or simple proteid compounds there is no occasion requiring them normally to be prepared to combine directly with the complex proteids. However when a foreign proteid is introduced into the general circulation by subcutaneous, intramuscular or other injections, these body cells are given the task of taking care of complex proteid. According to Ehrlich's conception of excessive production of receptors for which there is proper stimulus, the body cells will first produce receptors that break down or split the complex proteid so it can be assimilated by the tissue cells. These excessive receptors are the anaphylactic antibodies which exist both in the tissues and blood serum and provide a proteolytic agent able to catabolize the foreign proteid more rapidly and powerfully. It is now quite generally believed that the sensitized individual is sensitized because he possesses proteolytic substances resembling amboceptors (anaphylactic antibody or allergin) which break up the particular protein material so rapidly that toxic substances are formed and liberated in poisonous doses while in

the normal nonsensitized individual without excessive proteolytic substances the process of breaking down the proteid is so slow as to cause no intoxication. Heilner¹ has recently advanced the view that during the anaphylactic period the organism is unable to change completely substances that are usually metabolized with the greatest ease.

Serum sickness in most cases while undesirable and uncomfortable to the patient is far outweighed by the benefits derived from some of the antisera. As the indications and contraindications for injection of antisera are better understood by physicians fewer objections to serum therapy scientifically applied will be made. That serious objections to the use of immune sera have some foundation is evidenced by the fact that Rosenau and Anderson were able to collect data on a number of sudden deaths in man after the injection of immune serum. It has been noticed from the first that in the cases of sudden death following the injection of diphtheria antitoxin there is marked respiratory embarrassment. Rosenau and Anderson believe the essential lesion of serum anaphylaxis is localized in the respiratory centers. In collecting statistics on this subject these investigators have found two cases, "and also others have come to our notice," in which sudden death followed the injection of antitoxin into asthmatics. From these observations Rosenau and Anderson conclude that "the knowledge of the fact that injection of horse serum into asthmatics may be attended with danger should be considered in the use of antitoxins."

Auer² studying the hearts of rabbits succumbing to anaphylactic seizures due to horse serum, observed peculiar anatomic changes in those portions of the heart associated with its conduction process and furthermore has found that the auriculo-ventricular rhythm is disturbed in the fatal cases as well as in those recovering.

While there are some cases in which the injection of horse serum has been followed by sudden death due to hypersusceptibility to horse serum, most of the deaths have undoubtedly

¹Heilner: *Ztschr. f. Biol.*, 1912, LVIII, 332.

²Auer: *Ztschr. f. Physiol.*, 1912, XXVI, 363.

been due to other causes. Frequently physicians and laymen wrongly attribute undesirable conditions to the use of diphtheria antitoxin and other immune sera. In the use of some immune sera especially diphtheria antitoxin recovery from the disease is so rapid and early that the physician and patient overestimate the physical condition of the patient and as a result the patient is frequently allowed a certain amount of exercise. This at times is followed by unfavorable symptoms. Serum disease has been the cause of much agitation against the use of specific antisera in the treatment of and immunization against certain diseases. To overcome this prejudice it is necessary that the physician understand the indications and contra-indications.

Serum disease occurs more frequently after the second or subsequent injections than after the first. Furthermore if the interval between injections is less than ten days sensitization seldom occurs. Moss has advocated the testing out of individuals to be injected by first injecting one one-hundredth cubic centimeter of normal undiluted horse serum intradermically. If the patient is sensitized an inflammatory zone will appear at the site of the injection within twenty-four hours. Of course in many cases it is impossible to wait as long as this. It is a safe rule to give specific antisera only after a definite diagnosis has been made, to remember that there is some danger attending the injection of horse serum into asthmatics and that untoward effects of serum injections are more frequent when the interval between injections ranges from ten days to several years.

The indications for injection of immunizing doses are not clear or definite. Nurses and doctors however now rarely receive immunizing doses of antiserum. This is especially true of diphtheria antitoxin. The reasons for the abandonment of the practice of immunizing doctors and nurses are (1) that doctors and nurses are better able to avoid infection and so usually do not contract the diseases of their patients, and (2) the protection conferred by passive immunization is of short duration. Doctors and nurses because so frequently exposed to infectious diseases would have to be injected frequently to

be immune to the various infections of their patients. The custom concerning the immunization of individuals against possible infection because of exposure varies. It is the rule of some physicians upon the diagnosis of diphtheria in one member of the family to give all members of the family immunizing doses of diphtheria antitoxin. In some of the large children's hospitals and clinics diphtheria antitoxin is given to all patients on admission, this being done to avoid the outbreak of diphtheria epidemics. Such preventive measures hardly seem warranted. The custom of most physicians now is to give immunizing doses of specific antisera to such members of a family or household as are most likely to contract the disease; thus for example in a home in which there is diphtheria, diphtheria antitoxin is given only to the children. On this basis administration of antisera is justifiable, while indiscriminate immunization by the injection of serum is costly and may sensitize individuals to serum and later make the use of serum difficult or impossible when the injection of immune serum is definitely indicated. While it is true that some sera are ordinarily injected at intervals of two or three weeks without the production of serious results still it frequently happens that hypersensitization to serum develops to such a degree that passive immunization must be given up entirely.

SPECIFIC THERAPY DEPENDING ON CHEMICAL AGENTS.

For many years it has been known that certain chemical preparations are able to destroy micro-organisms. From this originated antiseptic surgery and cleansing and dressing of wounds and infected tissues with chemicals called antiseptics. As the body's own methods of combatting infection became known and it was realized that some of the substances the body provides for its own defense can be introduced into the body from without and that in this way recovery can be obtained, chemical means of destroying bacteria became less important. From time to time germicides, antiseptics and so on have however been advocated for the treatment of local infections.

The success of these has been slight because of three reasons: it is hard to supply the germicide to the infected tissue, it is difficult to supply sufficient chemical poison to kill the micro-organisms and not injure or destroy the body cells and most of the chemical substances having germicidal properties combine with the body tissues on administration and so lose their germicidal power. Until relatively recently only two chemical substances, quinine and mercury, have been recognized as being specific and efficient. The discovery of the specific therapeutic value of these two drugs was not the result of experimentation but was entirely accidental. More recently new chemicals and applications of old chemicals for specific therapy have been advocated. Efficient logical chemotherapy is beset with difficulties. Ehrlich's original conception of the side chains of the cell was that they served nutritive purposes but later studies have led him to believe that receptors for substances other than foods also exist. Of the receptors binding chemicals he believes that they are less complex and more firmly attached to the cell so that while new receptors are formed because of the binding of those existing on the cell, they remain sessile and are not cast off. It is well known that the body acquires a tolerance for certain things and likewise that micro-organisms may become nonsusceptible to certain chemicals. On the receptor basis these results might be due to atrophy and modification of receptors. These possibilities are of the greatest importance in chemotherapy for if doses so small are given that all of the micro-organisms are not killed "drug-fastness" may result. If this happens relapses occur and during each relapse there may be injury of structures of vital importance to the body. These are the results often obtained in the treatment of malaria, sleeping sickness, relapsing fever and syphilis. Ehrlich in full realization of these defects in chemotherapy has introduced his idea of "*therapia magna sterilisans*"—that is the use of chemicals which can be introduced in sufficient quantity to kill all of the micro-organisms at one time. This is the basis and fundamental principle of his "606" or "salvarsan" for the cure of syphilis.

In the application of chemotherapy the affinity between the

chemical and the protoplasm of the micro-organisms is of much importance. If the poisonous chemicals combine as well or more readily with the tissues and fluids of the body as with the parasites, the chemical is not suitable for therapeutic purposes. In the selection of chemicals it is of prime importance that they are not toxic or only slightly so for the host and markedly toxic for the parasite.

Antiseptic chemicals said to have therapeutic value are constantly being advocated by manufacturers. It behooves the physician to investigate these carefully and to get reports on the value of these drugs from the reliable medical journals rather than to base his decisions on the advertising literature furnished with the sample.

TREATMENT OF INFECTION WITH LEUCOCYTIC EXTRACTS.

Hiss and Zinsser believing that the leucocytes of persons suffering from infections have less phagocytic power than have the leucocytes of the normal individual, experimented with injection of extracts of leucocytes. The extracts were made from the fluid collected from the pleural cavities of rabbits that had 24 hours before received intrapleural injections of aleuro-nat suspensions. The exudate was centrifuged and the serum taken off after which the leucocytes were emulsified in water and allowed to stand at 37° C. for a few hours. Of this fluid 5 to 15 c. c. were injected several times. Injections of leucocytic extracts have been tried in various infections and the results have been favorable. The principal benefit seems to manifest itself in reducing intoxication. The treatment has not been applied generally but certainly warrants further clinical study.

TREATMENT OF INFECTIONS WITH NORMAL SERUM.

It has been known for a long time that the fresh normal serum of some animals has the property of dissolving the red blood cells of other species. Fodor, Nuttall, Buchner and

others observed that fresh normal serum can dissolve many species of bacteria. This dissolving power of normal serum is due to interbody and complement. Solution of bacteria is not specific, blood from one species of animal having interbody for various species of bacteria. The amount of interbody however is small as compared to that in the specific antisera and its action is not entirely specific.

More recently it has been found that normal serum is of therapeutic value in the healing of ulcers, wounds and so on and that it has some antihemorrhagic properties. Some of the applications of treatment with normal serum have been indiscriminate and on poor indications. Normal serum has been advocated by some for the treatment of infections when specific antiserum is not available or if a definite diagnosis of the causal organisms has not been made. As a basis for this some have assumed that the benefits derived from Bier's hyperæmia and so on are due to the action of normal serum. It must however be remembered that when there is infection the body makes a normal effort to overcome it and therefore the blood serum will contain some antibody so that hyperæmia resulting in accumulation or interchange of serum, furnishes more antibody to the area of infection. Recently reinjection of serum from the patient's own blood has been tried. In some cases fresh serum has been used while in others the serum has been inactivated by heat before injection. Good results have been claimed for this autoserotherapy and probably the field of application will be increased considerably.

There undoubtedly are definite indications for the use of normal serum and benefits are derived from its use in some cases but in all infections it is best to identify the infecting organisms and use specific treatment when possible.

CHAPTER V.

SPECIFIC DIAGNOSIS AND TREATMENT IN THE DIFFERENT INFECTIONS.

In the previous chapters the various symptoms and signs of infections, their course and general methods of diagnosis and treatment have been outlined and considered. Therapy for many of these is specific. In some cases attempts are made to simulate the natural processes of defense while in others specific drugs and chemicals are available. Before specific treatment can be instituted it is necessary that a diagnosis of the etiological factor be made. To make a specific diagnosis laboratory methods are most valuable but by the competent physician the results of laboratory tests are only regarded as and given the weight of cardinal symptoms. It is taken for granted by the author that the clinical symptoms are known to the physician so that little space is given to clinical symptoms and signs. To the laboratory tests more space is given because they and their interpretation are less well known to the physician. Laboratory tests and examinations are subject to error and at times give wrong impressions just as do clinical symptoms and signs and histories of the patient and the disease.

Among the different investigators there are differences in the details of the methods employed in specific diagnosis and treatment. When making use of the methods given here the reader is again referred to the general methods of diagnosis and therapy given in the earlier chapters.

INFECTIONS WITH THE DIFFERENT VARIETIES OF MIC. PYOGENES.

Staphylococci are the most common pus producers in man. Because of their wide distribution and frequency of occurrence on the surfaces of the body they very often cause and complicate diseases. They produce disease in almost all parts of the body, the infection may be local or general, primary or secondary, may follow injury

to the tissues or produce disease on apparently normal and healthy surfaces.

Differential Specific Diagnosis.—CLINICAL.—The infections and diseases produced by staphylococci vary clinically, pathologically and anatomically. There are however some characteristics which offer aid in diagnosis. Of these the most important local symptoms are pus formation to the point of fluctuation and great pain and swelling of the regional lymph glands. The four cardinal symptoms of infection are present in all local staphylococcus infections. In infections of the serous surfaces such as peritoneum, staphylococci are most prone to produce sufficient inflammatory reaction to localize the infection. Staphylococci are less likely to cause septicæmia and metastatic abscesses than are the streptococci.

BACTERIOLOGICAL DIAGNOSIS.—This is best made by cultures from material from abscesses and other lesions. An enriching medium is seldom necessary, plates or slants of agar being sufficient. If cultures are to be obtained from the blood it is usually necessary to inoculate bouillon in flasks and after 24 hours of incubation to make transfer cultures from this bouillon. To determine the variety of *Mic. pyogenes* potato slants are best. *Mic. pyogenes aureus* produces a golden or brownish-yellow growth, *Mic. pyogenes citreus* a lemon yellow growth and *Mic. pyogenes albus* a white growth.

In many cases stained specimens of the pus will give the diagnosis of staphylococcus, the organisms occur in irregular arrangements, sometimes in pairs and always are Gram positive.

SERUM DIAGNOSIS.—Opsonic index determinations for a time were regarded as being of value not so much in the diagnosis of staphylococcus infection as in the determination of the progress of development of immunity. For diagnostic purposes the opsonic index is of little value.

Agglutination tests for the diagnosis of staphylococcus infections have been little resorted to. In most cases it is possible to demonstrate and identify the organisms directly. Agglutination tests have however been of value in distinguishing pathogenic from saprophytic staphylococci. These tests have tended to show that pathogenic staphylococci are not as widely distributed in nature and on the surfaces of the body as was formerly supposed. This

has further been substantiated by the failure of the saprophytic staphylococci to produce hæmolysin and leucocidin.

Immunity and Specific Therapy.—It is hard to determine the natural immunity of man to staphylococci but apparently it is considerable. Artificial immunity in animals is quite easily obtained but in some animals attempts at immunization result in amyloid degeneration. With the development of active immunity phagocytosis is increased but this is not conclusive evidence that bacteriotropic substances or opsonins play the entire part in protection. If leucocidin is injected antileucocidin develops, and if the staphylococcus hæmolysin is injected antihæmolysin is produced. Agglutinins also are produced in artificial immunization.

Vaccination and Active Immunization.—The various infections caused by the *Micrococcus pyogenes* group have been considered most satisfactory for vaccine treatment. The list of staphylococcus infections treated successfully by bacterial vaccines includes boils, carbuncles, acne, sycosis, felons, styes, septic wounds, middle-ear infections and in fact most of the infections which remain localized. The results obtained in treatment with killed cultures have varied. Probably the best results have been obtained in localized surface infections. In the treatment of these cases it has been found that autogenous vaccines are not necessary. It is however important in all cases to determine the species and variety of organisms causing the infection and to use this variety in the treatment. The staphylococcus vaccines with which the writer has obtained the best results are those made from different isolations of the corresponding variety of organisms. Thus a *Mic. pyogenes aureus* stock vaccine which has given good results is one made from three isolations, the organisms being obtained from the lesions of chronic marked furunculosis, from an acute boil on the face and from a patient having multiple pustules on the back of the neck. Most patients suffering with furuncles and carbuncles show a marked improvement within twenty-four hours after injection and some of these patients remain well after that. Everyone who has treated patients suffering with furuncles or carbuncles must have observed that there occasionally is recurrence of these conditions after apparent recovery following upon the injection of suitable vaccines. If Wright's method of vaccination according to the opsonic index is

followed there are apparently more recurrences than when the injections are given at intervals not less than five days nor greater than ten days. In all cases where there is pus the best results are obtained if a small incision is made twenty-four hours after vaccination. This relieves the pain and makes it possible to remove the phagocytes which have ingested the bacteria. For several days the pus should be drawn off through this incision. The advantage of drawing off the leucocytes when they have ingested the staphylococci is evident when it is remembered that staphylococci secrete leucocidin which destroys leucocytes. Artificial hyperæmia as by Bier's method, massage and so on are of great importance when there is stagnation.

The dosage of staphylococcus vaccine varies but usually adults receive one hundred million to three hundred million killed cocci at first. After this the doses are increased, injections being made at intervals of from four to ten days. As was mentioned before the author has had fewer relapses when at first six hundred million cocci were injected while the later doses were smaller, at times only one hundred million cocci.

Because staphylococci are so widely distributed and play a part in various infections, mixed vaccines containing staphylococci have been much advocated. In this connection it is to be remembered that in active immunization it is usually desirable that the immunization be against all of the species present in the disease. This implies that immunization against staphylococci should be attempted only when staphylococci play a part in the disease so that the diagnosis of staphylococcus infection is necessary before immunization against it is to be attempted. Furthermore mixed infections are at times more easily recovered from after the infection with one of the species of micro-organisms has been overcome. Rather than use mixed vaccines indiscriminately autogenous vaccines and mixed vaccines based on the bacteriological diagnosis are to be preferred. This holds true for gonorrhœa, sycosis barbæ, acne, tuberculosis and so on.

Acne.—Staphylococcus vaccines of various kinds have been used in treating this disease. Acne vulgaris has been successfully treated by vaccination only in certain cases. The value of bacterial injections in this condition was so emphasized by Wright that for a

time it was the common belief among clinicians that vaccinations with killed cultures of *Micrococcus pyogenes albus* were applicable in all cases of acne. This however is not the case. In the lesions of many patients suffering with acne vulgaris the white skin coccus cannot be found. In such cases either the species of organisms causing the pustules must be injected or else vaccine treatment should not be applied. The work of Sabouraud, Gilchrist, Fleur-ing and so on has shown that other organisms than the white skin coccus play a part in acne and better results have been obtained in many cases where a vaccine made of the acne bacillus has been used. Because of difficulties in growing this organism physicians seldom make their own acne vaccine.

When the vaccine treatment is used other means of treatment must not be discontinued. Incisions and evacuation of the pustules, cleansing of the skin with benzine, expression of comedones or blackheads, the bathing of the infected part in hot water, application of sulphur or other suitable lotions and the regulation of the diet are all of assistance to overcome this disease. Even under these measures the treatment is not always successful.

Serum Therapy and Passive Immunization.—Various anti-staphylococcic sera have been made. They are usually obtained from horses and other animals that have received repeated injections of dead and living cultures of these organisms. The method of its action is not clearly understood although any protective value it may have is probably due to lytic and opsonifying power. The value of the serum as it can now be obtained is inconsiderable and its injection in the treatment of staphylococcus infections is seldom or never warranted except in septicæmia, peritonitis and so on. Then from 10 to 20 c. c. are injected at a dose, but larger quantities may be given.

Prophylaxis.—Prophylaxis against staphylococci is especially important in surgery and concerns itself principally with asepsis and antisepsis. In cases of furunculosis, acne vulgaris, sycosis barbæ and other staphylococcus infections thorough cleansing of the skin about the infected area is of great importance. Immunization against staphylococci before operation has been suggested by some but it is hardly fair for a surgeon whose technique

is such that previous immunization is necessary, to expose a patient to an operation.

INFECTIONS WITH THE DIFFERENT VARIETIES OF STREPTOCOCCI.

Streptococci or cocci in chains were first recognized by Ogston and Fehleisen and later by Rosenbach as the causes of various serious diseases in man. Streptococci are widely distributed but not nearly all of them are pathogenic. For a time efforts were made to distinguish between pathogenic and saprophytic varieties on the basis of the length of chains—those consisting of eight or more cocci being regarded as pathogenic to man and those of six or less as saprophytic. The streptococci pathogenic to man are probably of different species, the best known being *Streptococcus pyogenes*, *Streptococcus erysipelatis*, *Streptococcus rheumaticus*, *Streptococcus viridans* and *Streptococcus scarlatinæ*. They are ranked with the most virulent of pathogenic bacteria. The species name in some cases indicates the diseases from which they are isolated. The principal diseases in which they are found are wound infections, cellulitis, septicæmia, metastatic abscesses, rheumatism, scarlet fever, tonsilitis and endocarditis. Because of the difficulties in distinguishing the species and varieties at least as far as the physician is concerned, only the clinical conditions streptococcus infections, erysipelas, rheumatism and scarlet fever are here considered. Of these, streptococcus infection may be primary or secondary, simple or mixed. Any part of the body may be infected by them.

Differential Specific Diagnosis.—CLINICAL.—Streptococcus infections usually start in wounds or injuries but the initial infection may also be located in apparently normal tissues as the tonsils, mucous membranes, and so on. The regional glands are usually enlarged and tender. In infections of the extremities usually the lymphatics draining the infected area are red and tender. Infections of the tonsils are frequently followed by rheumatism and endocarditis. Early in the infection especially when the peritoneum is involved the pulse rate is high while there is only slight fever, but later the temperature rises markedly. Patients with general streptococcus infection frequently remain conscious and rational

up to a short time before death. Streptococci are prone to appear in the form of a septicæmia and to produce metastatic abscesses in the internal organs. Streptococcus peritonitis is characterized by only small amount of exudate and marked congestion of blood vessels of the visceral and parietal peritoneum.

BACTERIOLOGICAL DIAGNOSIS.—Demonstration of streptococci may be made from cultures or stained specimens from smears. In isolating and cultivating streptococci it is to be remembered that they do not grow well on artificial media, that they grow slowly and in small colonies, that there are numerous species and varieties, and that some of these are saprophytic. In all cases when isolations are to be made blood or blood serum, hydrocele or some body fluid should be present in the medium, the cultures should be incubated for at least 24 hours at 37° C. and then examined closely. Inasmuch as blood stream infections are common blood cultures are of great assistance in the diagnosis. The organisms are quite easily isolated from the vesicles, pustules and the border of the inflamed area in erysipelas, material from the smaller and clear vesicles being preferred. When cultures are made from the tonsils the technique usually is that followed for the diagnosis of diphtheria bacillus infection. In rheumatism the part is cleansed as for operation and with a sterile hypodermic outfit fluid from the joint is obtained and used for the inoculation of the medium. Separate isolations are tested on the different culture media for cultural characteristics, staining properties and morphology. (See p. 35.)

Stained specimens of pus give an early diagnosis in most cases. The organisms are Gram positive. Sometimes the chains are very short and may not be diagnosed as streptococci but if inoculations of bouillon are made definite chains will be formed.

SERUM DIAGNOSIS.—The bacteriological diagnosis of streptococcic infections is usually so simple that serum diagnosis is seldom resorted to. The opsonic index has been used to some extent to determine the progress of immunization. Agglutination, fixation of complement and other tests are seldom resorted to because they are not often necessary.

Immunity and Specific Therapy.—One attack of the usual streptococcus infection apparently confers little protection against subsequent infections, in this way differing markedly from the in-

fection in scarlet fever which leaves an almost certain immunity. In recovery from these infections antibodies must play their part but apparently they remain active only a short time. While the pathogenic streptococci cause marked intoxication in man and some of the animals, on culture media they produce little toxin. The killed bacteria likewise are little toxic.

Vaccination and Active Immunization.—Various kinds of streptococcus infections as arthritis, pneumonia, pericarditis, erysipelas, local infections and endocarditis have been treated by vaccines. Usually these infections are acute and severe and it often is a question whether sufficient time for the production of immune bodies as a result of vaccination will elapse before death or spontaneous recovery terminates the disease. At times striking results have been obtained while in other cases not treated by vaccination there have been equally striking recoveries. One needs only to remember the cases of streptococcus infections of the uterus, of septicæmia and erysipelas that have recovered in a few days without any specific treatment to realize that it is easy to attribute beneficial results to the use of streptococcus vaccines in cases in which the same results might have been obtained without them. When there is much intoxication vaccines are seldom indicated, the anti-streptococcic sera being of more value at this time than the vaccines.

Streptococcus vaccines apparently are of value at times. This is especially true in sub-acute and chronic cases and applies to infections in almost all parts of the body. Wright and others have reported successful treatment of streptococcus septicæmia by the injection of streptococcus vaccine. It must however be remembered that streptococcus septicæmia is at times recovered from even when no specific treatment is given.

In all cases it seems best to make an autogenous vaccine because the various strains of streptococci have special and specific action and therefore cannot be used indiscriminately. To overcome this, mixed streptococcus vaccines are on the market but autogenous vaccines are to be preferred to these. Various investigators have found that vaccines in which the streptococci are killed by galactose instead of by heat have greater therapeutic value. The dose injected varies from ten to one hundred million cocci injected at intervals of from three to twenty days.

Erysipelas cannot be produced by all of the streptococci nor will all streptococci isolated from erysipelas produce the disease. The portal of entry of the organisms to the lymph spaces and interspaces in the connective tissue is not always clear. In 1907 the author treated 37 patients suffering with erysipelas by injections of killed cultures of streptococci isolated from the lesions of erysipelas. From the results obtained it was impossible to determine the value of the injection of the killed cultures of streptococci. While the injection of from twenty-five million to one hundred million cocci did not prevent migration and recurrence, the apparent shortening of the duration of the disease suggests that streptococcus vaccines are of some value. The effect upon the duration of the disease was found to be uncertain because during the time of the investigation no suitable cases remained untreated with which comparison could be made. It is known that the duration of the disease varies in different years. Ross and Johnson have reported on the treatment of erysipelas with a vaccine made from *Streptococcus erysipelatis* and conclude that such vaccine exercises a specific and controlling influence on the course of the disease. These investigators make initial injections of from ten to twenty million killed streptococci and repeat these injections every day or two according to the clinical results obtained.

The relation of streptococci to scarlet fever is not definitely established. Streptococci undoubtedly frequently are found on the tonsils and even invade the blood stream producing septicæmia in scarlet fever but they probably play the role of a secondary or accompanying infection. If this is true we may still expect benefit from streptococcus vaccine and serum therapy. While some investigators believe that the streptococci isolated during the disease show such distinctive characteristics as to deserve the name of *Streptococcus scarlatinae*, most bacteriologists are not agreed to it. If active immunization is to be undertaken it is however probably advisable to make an autogenous vaccine or at least one from streptococci isolated from scarlet fever. The curative value of active immunization by the injection of killed cultures is still doubtful but the results of protective vaccination as published by Gabritchewski and other Russians are encouraging. The usual doses for streptococci are given.

Specific treatment of articular rheumatism has been retarded to some degree because the etiology is still obscure. There are no certain clinical and pathological features, few people die during the disease so that even the diagnosis is uncertain. In the condition we may call rheumatic fever, streptococci have been found more frequently than have other organisms. For a time these streptococci were named *Micrococcus* or *Streptococcus rheumaticus* but from the work of Cole and Meakins, Harris and others *Mic. rheumaticus* cannot be differentiated from *Streptococcus pyogenes*, although the lesions produced in man have special characteristics. The disease frequently follows streptococcic tonsilitis. Active immunization by injections of killed cultures has been of value in many cases. The vaccine should preferably be an autogenous one made from the cultures isolated from the blood, joints or tonsil. Administration of the streptococcus vaccine should be made with caution, not during the acute stage of the disease but after the severe symptoms have subsided. Horder¹ has recently emphasized the importance of preceding vaccine treatment by the necessary local treatment as drainage, curettage, and so on.

Serum Therapy and Passive Immunization.—Antistreptococcic serum was first made by Marmorek in 1895. This investigator immunized horses by injections of increasing doses of living virulent streptococci. With serum from these animals he was able to confer passive immunity to rabbits and also made attempts to treat erysipelas and puerperal fever in the human. Denys and LeClef have immunized animals with bouillon cultures of streptococci and claim to get favorable results upon injection of serum from the immunized animals. Van der Velde, Denys and others, believing there are different strains of streptococci and that the antisera produced are specific for the especial strain used in immunization, immunized animals with the different strains of streptococci thus making what is called a "polyvalent" antistreptococcic serum.

The sera which are now on the market are almost universally made by immunizing horses with repeated injections of increasing doses of killed and later living bouillon cultures of numerous strains of streptococci recently isolated from patients. The essential difference between the various antistreptococcic sera are that Marmorek

¹Horder: *Lancet* (London), 1912, CLXXXII, 1053.

immunizes animals with only one strain of streptococci the virulence of which he has increased by passages through animals, Tavel uses various strains of streptococci whose virulence is slight, Aronson uses virulent and avirulent strains isolated from man and Ruppel uses a number of isolations all of which are injected both from cultures and after passage through animals. Several months are required to immunize horses from which the immune serum is to be obtained. After testing the serum for sterility it is tubed in suitable syringes.

Standardization and determination of the amount of protective substances in antistreptococcic serum has not been successful because the susceptibility of animals to streptococci varies and because the method of protective action of the serum is little known. Usually the dose injected is regulated according to cubic centimeters of polyvalent antistreptococcic sera. *The potency of this serum decreases relatively rapidly* so that it ought not to be used later than six months after the bleeding of the immunized horse.

The method of action of antistreptococcic serum is little understood. The serum contains agglutinins but these have not been regarded of value in immunization. With the discovery of bacteriotropins by Neufeld and Rimpau and opsonins by Wright and Douglas new light has been thrown on the action of these sera for it has been found that in antistreptococcic serum there is present a specific substance which increases and stimulates phagocytosis of the streptococci.

Antistreptococcic serum has been used especially in septicæmia, local infections following traumatism, streptococcic pneumonia, meningitis, rheumatism, erysipelas, puerperal sepsis, scarlet fever, secondary infection complicating tuberculosis and in fact all streptococcus infections. The serum method of treatment is to be preferred to vaccine treatment in acute infections and when there is much intoxication. In urgent cases intravenous injections probably yield the earliest results.

In the usual streptococcus infections polyvalent sera are used, Marmorek's univalent serum has apparently not been as satisfactory. In erysipelas Koch and Petruschky found the serum to have no protective value. Various other observers have claimed a curative value for antistreptococcic serum. Weaver and Tunnicliff recently

have reported a falling of temperature, decrease of local symptoms, disappearance of the delirium and marked general improvement in excess of what could be expected in the usual course of the disease.

Treatment of scarlet fever by the injection of antistreptococcic serum has been practiced for some time. Of the different antistreptococcic sera used in scarlet fever most has been claimed for Moser's serum obtained from horses immunized to about twenty strains of streptococci cultivated from cases of scarlet fever. Escherich, von Pirquet and Schick, Moser and others believe there is a shortening of the course, reduction of fever and general improvement by the use of Moser's serum. Baginsky makes the same claim for Aronson's serum. Schick¹ in a recent article refers to the good results from the use of the serum by the Russians. He states that in mild cases no serotherapy is needed but that it should be reserved for the dubious and severe cases, especially those in which there is much intoxication. The great drawback to the serum is the large quantity necessary, 200 c. c. for an injection and 100 c. c. for children under 18 months old. The use of the serum does not bind us to accept streptococci as the etiological factor in the disease but rather to regard streptococci as causing complications. That Moser's serum necessarily produces better results than antistreptococcic serum is not universally accepted.

In rheumatism most has been claimed for Menzer's serum obtained from animals immunized by injections of various strains of streptococci obtained from the tonsils of patients with rheumatism. Other antirheumatic sera are made by immunization to streptococci from the joints of patients having rheumatism. The serum is only indicated when there is severe intoxication while during the subacute and chronic stages the vaccine is probably better.

The therapeutic and prophylactic results obtained by the use of antistreptococcic serum vary considerably and are apparently dependent on various conditions such as grade and kind of immunizing serum, day of disease on which injections are made, amount of serum injected, virulence of the infecting organisms and determination of the invading organism. From the various reports which have been received from clinicians who have used these sera there can be no doubt as to the beneficial results which may at times be

¹Schick: *Therap. Monatsch.*, 1912, XXVI, 258.

obtained from their use. In order that good results may be obtained however it is essential that it be definitely determined that the streptococcus is responsible for the diseased condition, that a high grade of fresh polyvalent serum be given early in doses varying from 10 to 100 c. c., and that these injections be repeated at intervals of from four to eight hours.

After injection of antistreptococcic serum the patient usually rests more quietly for several hours. This however need not indicate that recovery will follow. The therapeutic effects of the serum are manifested by the relief of symptoms, decline in fever, improvement of the pulse and subsidence of the nervous symptoms. These effects usually appear within twenty-four hours after injection if the serum is to be of value and if no relief comes within twenty-four hours after two injections of from 20 to 100 c. c. of serum, no beneficial results are to be hoped for. No untoward effects of the serum are met with except the occasional skin rashes which have already been discussed elsewhere.

Antistreptococcic serum even though its curative results are uncertain ought to be used in every case of severe acute streptococcus infection. The use of the serum in scarlet fever is not universal but probably should be resorted to in the severer cases. In the chronic cases of discharging sinuses and so on streptococcic vaccines are at times used to greater advantage than antistreptococcic sera.

The measures to *prevent* infection with streptococci concern themselves principally with cleanliness. Immunization by active or passive methods is apparently of little value because of the short duration of immunity to these organisms.

INFECTIONS WITH MICROCOCCUS GONORRHÆÆ.

The establishment of *Mic. gonorrhææ* as the etiological factor in gonorrhœa was made by Neisser in 1879. The animals are not susceptible to the disease but man is most susceptible. The portal of entry is found principally in the mucous membrane. From here the organisms invade other parts of the body preferring glandular tissue but also enter the blood stream through which they are carried to the various parts of the body causing lesions in the heart valves, in the articular surfaces and so on. Intoxication occurs in most of

the infections during the acute and sub-acute stages of the disease. In the chronic stages patients often experience no symptoms whatever.

Differential Specific Diagnosis.—CLINICAL.—The clinical diagnosis of gonorrhœa is usually easy in the acute urethral and vaginal infections because nearly all of these are caused by the *Mic. gonorrhœæ*. Infection with gonococci is characterized by intense inflammation, pain and prostration. This holds true in infection of the peritoneum, conjunctiva and so on. Gonorrhœal arthritis in a large number of cases involves only one joint at a time and again presents an intense inflammatory reaction.

BACTERIOLOGICAL DIAGNOSIS.—This offers the best means of positive diagnosis. In the examination of secretions from the genito-urinary tract examination of smears stained by Gram's method are sufficient. The organisms do not hold the Gram stain and are found arranged in diplococci and usually in polynuclear cells. When there is peritonitis or arthritis cultivation of the organism is of great importance. It must be remembered in this connection that the organism is a strict parasite so that human blood, ascitic or other body fluid is necessary in the medium. When there is septicæmia blood cultures should be made. In sub-acute and chronic infections other organisms, especially staphylococci, may take a part in the disease. This is diagnosed in the usual manner.

SERUM DIAGNOSIS.—Because of the difficulty experienced at times in demonstrating gonococci in the secretion of chronic infections, tests to demonstrate the presence of antibodies have been introduced. Torrey and others have shown that on immunization of animals agglutinins, bacteriolysins, precipitins and complement deviation bodies are produced. Tests for these antibodies in man during and after infection have been partly successful but practically all of the tests are so surrounded with difficulties in technique that they are hardly possible for routine diagnostic purposes.

In 1909 Bruck described a method for an allergy reaction. In this country Cole and Meakins and Irons reported a cutaneous reaction in gonococcal infection. Irons applied the allergy test by making glycerin extracts of autolyzed gonococci with which he injected the skin of the arm. The value of the reaction is not defi-

nately determined, some reaction occurring in healthy individuals as well as in patients suffering with infection with diplococcus intracellularis and *Mic. catarrhalis*. London has injected cutaneously small amounts of gonococcus vaccine and in positive cases has obtained a reaction consisting of an erythema one to three inches in diameter in the center of which there is a small red papule in from 12 to 24 hours.

Immunity and Specific Therapy.—Experimental antibodies have been produced in some animals, likewise antibodies are present in the serum of the human at some time during infection. However in man infection with the organisms does not produce an immunity to subsequent infection. *Mic. gonorrhææ* does not produce a soluble toxin but the cocci themselves are toxic. Endotoxin may be obtained in bouillon cultures after filtration. This is toxic to man and animals producing an inflammatory exudate on the mucous surface, enlargement of the neighboring glands, fever and if large enough doses are given intraperitoneally to the guinea-pig death will result. Animals may be immunized to these toxins but practical application of this to humans has met with little success.

Vaccination and Active Immunization.—Theoretically the treatment of gonorrhœal infections with vaccines seems practicable. However vaccines have been of little value in treatment of acute urethritis or any of the acute gonococcus infections. In the sub-acute and chronic infections of the various parts of the body the results have been attended with considerable success. The vaccines used have been autogenous and from stock cultures. Based on the studies of Cole and Meakins and others a mixed vaccine of several strains is as satisfactory as an autogenous vaccine. Isolation and cultivation of *Mic. gonorrhææ* is attended with much difficulty so that autogenous vaccines are not easily made. The doses injected vary but usually the smaller ones (twenty to fifty million in acute cases and fifty to one hundred million in the chronic cases) are preferred if clinical reactions are to be avoided. Injections are repeated at intervals of three to ten days. When there is a mixed infection the organisms causing the secondary infection should be determined and a specific vaccine for these organisms made or obtained rather than to resort to the "shot-gun" vaccines supplied by some of the manufacturers.

Serum Therapy and Passive Immunization.—Various attempts have been made to produce an antigenococcic serum for passive immunization of man. It is a well known fact that in the human no immunity results from an attack of gonorrhœa and for this reason, together with the lack of favorable results from the use of antigenococcic sera, most investigators have concluded that a curative immune serum for *Mic. gonorrhææ* cannot be produced. In most of the attempts at the production of antigenococcic serum old cultures of the organism have been used.

In 1906 Torrey in an attempt to make antigenococcic serum made the observation that the toxin of *Mic. gonorrhææ* present in old fluid cultures is toxic for the small laboratory animals. The toxin of the gonococcus has been studied at various times and has been generally regarded as being derived from the dead and disintegrated bodies of the organism, though by some it has been claimed to be a true extracellular toxin. Torrey found that it is not possible to immunize the small laboratory animals to this toxin but that in fact at times a true hypersusceptibility may develop as a result of injections of this toxin. This investigator observed however that animals can be immunized to the living and dead organisms of gonorrhœa and that in the blood of animals so immunized agglutinins and lysins are present. Based on these observations Torrey holds that the efficiency of the serum depends upon specific bactericidal substances which act because of lytic rather than of opsonifying power. Phagocytosis according to this investigator is of little importance in the destruction of gonococci.

The method of preparation of the serum of Torrey and Rogers as perfected and recommended by the investigators and used by the different serum producers is as follows: strong, healthy rams receive intraperitoneal injections of increasing amounts of twenty-four hour old ascitic agar cultures of various recently isolated, virulent strains of *Mic. gonorrhææ*. The culture is suspended in salt solution and for the first two injections this suspension is heated to 65° C. for one-half hour before the injection is made. Nine or ten injections are usually necessary to produce serum of high value. After immunization is completed the animal is bled from the carotid arteries and the serum allowed to separate out. After this the serum is collected, filtered and tested for sterility. Immunization is made in

rams because blood from these animals is apparently little toxic to the human. Polyvalent serum is used because the immune bodies for one strain are specific for that strain and inasmuch as the strains of gonococci vary in the different infections, immune bodies for all strains must be present in the serum.

Standardization of this serum has not been effectual. It seems quite definite that it possesses no antitoxic or opsonic value but is dependent for its efficiency on its lytic action. Because of a lack of any better method of determining the immunizing value of this serum, determinations have usually been made of its agglutinating value. The amount of serum injected is based on cubic centimeters rather than units of immune substance.

The method of treatment with antigonococcic serum consists of injections of 2 to 6 c. c. of serum at intervals of from one to four days as may be indicated clinically. The injections of serum are made in the loose subcutaneous tissue in convenient parts of the body. In every case treated with antigonococcic serum all the other methods known to be of value in the treatment of the particular condition should be employed. Antigonococcic serum seems to cause serum disease more frequently than most of the other immune sera. In all cases it is to be remembered that because this method of treatment frequently must be carried over a long period of time, serum injections must be made at intervals of not more than seven to eight days.

Antigonococcic sera have been tried in the treatment of the various infections by *Mic. gonorrhææ*. The value of the serum has varied much. The greatest value seems to be manifested in the severer complications of gonorrhœal urethritis as septicæmia, endocarditis, peritonitis, meningitis and arthritis. For the acute urethral, vaginal and conjunctival infections it is of little value. It is possible that where beneficial results are obtained it is because of active instead of passive immunization. Active immunization of animals to gonococci probably yields little antibody of value in the passive immunization of man because animals are not susceptible to the action of the organisms. Antibody formation is a part of the normal defense of the body and if the organisms do not produce

disease in animals we can expect little formation of antibody. Wright some time ago pointed out that sera may contain antigen. If the animals that are to furnish the antiserum are immune to the organisms and do not respond by antibody production they probably also do not bind the organisms or their endotoxins and therefore these may occur in the serum. In this case the serum may act as a vaccine instead of an antiserum.

Prophylaxis.—Prevention of infection with gonococci is largely one associated with the control of prostitution as most of its infections occur during coitus. The use of antiseptics immediately after sexual intercourse is of great importance in preventing infection. Protective immunization is of no practical value.

INFECTIONS WITH MICROCOCCUS PNEUMONIÆ (DIPLOCOCCUS LANCEOLATUS).

Micrococcus pneumoniae was first definitely recognized and described by Sternberg, Pasteur, Weichselbaum and Fränkel and to the latter two belongs the credit of association of the organism with lobar pneumonia. The principal disease caused by the pneumococcus is lobar or croupous pneumonia. Pneumonia or inflammation of the lung may be caused by a variety of micro-organisms, the principal ones being *Diplococcus pneumoniae*, *Streptococcus pyogenes*, *Micrococcus pyogenes*, *Bact. pneumoniae* (Friedländer's bacillus), *Bact. influenzae*, *Bact. diphtheriae*, *Bac. coli*, *Bac. typhosus*, *Micrococcus catarrhalis*, *Bact. pestis*, and *Bact. tuberculosis*. While all of these organisms may cause pneumonia, pneumonia frequently occurs only as a complication in diseases produced by these bacteria. Pneumonia produced by pneumococci is of the lobar type in 80 to 90 per cent. of the cases but it may be of the lobular or acute interstitial variety. Pneumococci not only produce disease of the lungs but may cause infection in the various parts of the body producing otitis media, conjunctivitis, bronchitis, pleurisy, peritonitis, meningitis, endocarditis, salpingitis, and so on. In many cases pneumococcus infection is complicated by infections with other micro-organisms, the principal ones being *Streptococcus pyogenes*, *Micrococcus py-*

ogenes, *Bact. tuberculosis*, *Bac. coli*, and so on. Pneumococci occur in the nose, mouth and pharynx of many apparently well individuals. During and after convalescence from pneumonia they frequently persist. They are often found in the conjunctiva and may occur in the stomach and intestine in well people.

Differential Specific Diagnosis.—CLINICAL.—Pneumococci are not limited as etiological factors to disease of the lungs nor are all pneumonias of pneumococcic origin and even where pneumococci are the cause of pneumonia the course is frequently influenced and modified by the presence of other micro-organisms.

True acute lobar pneumonia in 80 to 90 per cent. of the cases is a pneumococcus infection and in most cases terminates by crisis between the third and twelfth days of the disease, at which time the leucocyte count usually also decreases. Pneumococci in most infections produce severe intoxication and are to be classed with the bacteria producing the highest leucocyte count during the period of infection. Pneumococcus arthritis frequently involves more than one joint, has a tendency to localize in joints already the seat of chronic infection, the effusion is purulent and recovery is slow usually leaving an ankylosis.

BACTERIOLOGICAL DIAGNOSIS.—Bacteriological diagnosis is not frequently resorted to in the regular acute cases of lobar pneumonia. In pneumonia not of the regular type which terminates by crisis, in infections of tissues and organs other than the lung and in all cases where specific vaccine or serum therapy is to be used, bacteriological diagnosis is absolutely essential. In the bacteriological diagnosis considerable difficulty is met with for not only are the isolation and cultivation of the organisms associated with difficulty but the differentiation by cultural and morphological characteristics is difficult and surrounded by contentions of the various investigators. Even when the organisms are definitely diagnosed their mere presence does not speak absolutely for their etiological importance. Generally it is accepted that the organisms occur in pairs, hold the Gram stain and have a capsule which is

present when the organisms occur in the tissues or are grown in milk or serum media. Hiss has shown that most pneumococci produce acid on inulin, and Schottmüller, Rosenow and others have found that they do not hæmolyze red blood corpuscles. When a diagnosis of the organism in pneumonia cannot be made from the clinical picture and properly stained slides, subcutaneous inoculation of mice or rabbits with the sputum should be made. If pneumococci are present the animals will die in 24 to 72 hours, the blood in all cases shows a severe septicæmia and the organisms are demonstrable in stained smears of the blood.

In pneumonia blood cultures have been taken by various investigators. Fluid medium has given the most satisfactory results especially when a small piece of calcium carbonate is added to neutralize the free acid formed. The general results of blood cultures show that positive cultures are obtained more frequently in fatal cases and that after lysis occurs positive cultures are rare.

SERUM DIAGNOSIS.—This offers little assistance and is seldom resorted to. Agglutinins are formed to some degree at the time of or near the crisis but seldom is agglutination positive in serum diluted more than fifty times. During the disease agglutinins, precipitins, lysins and opsonins are produced but seldom in excessive amounts.

Immunity and Specific Therapy.—As a result of a successful combat with pneumococcus infection the individual receives some degree of immunity. The duration of this immunity is variable and in all cases probably short. Attempts to artificially immunize animals to the pneumococcus have been partially successful. The organisms do not produce an extracellular toxin so that antitoxic immunity cannot be produced in animals as a result of artificial immunization.

The nature of the intoxication in lobar pneumonia and other infections with pneumococci has been studied. Cole has suggested the possibilities that the intoxication may be due to disturbances in the metabolism of the body, the pneumococci merely being an exciting factor, or that the intoxication may arise from the breaking down of the exudate at the seat of infection. He and most others have however given much

study to the production of poisons by pneumococci within and outside of the body. Concerning toxins produced outside of the body, that is in cultures, there is much difference of opinion. These toxins have been obtained in different ways and possibly different poisons have been obtained by the various methods. F. and G. Klemperer prepared endotoxins by precipitation with alcohol. Rosenow who has made extensive experiments on the toxins and methods of immunization to pneumococci has found that when highly virulent pneumococci are allowed to autolyze in a sodium chloride solution there appears at a certain period a highly toxic substance. On injection of large enough doses of toxic material and autolyzed pneumococci he observed paralysis of the mechanism of immunization. The highly toxic substance in pneumococcus autolysate he found is soluble in ether and so can be separated from the other parts of the autolysate. This highly toxic substance did not call forth immunizing substances. Cole has dissolved the bacteria in a dilute sodium cholate solution and has found that this extract is extremely toxic when injected into guinea-pigs and rabbits and further, that the symptoms produced are like those in acute anaphylactic shock.

Vaccination and Active Immunization.—Recovery from pneumococcus infection especially from lobar pneumonia indicates an acquired active immunity. The duration of this immunity however is short. Fränkel in 1886 increased the resistance of susceptible animals by injections of dead and later living virulent cultures. Active immunization will probably never be of great value in the majority of cases of lobar pneumonia due to pneumococci because the crisis comes on about the time the results can be expected from the vaccination. The results of simple vaccines (pneumococci killed by heat) in lobar pneumonia have not been uniform. Wadsworth¹ has emphasized the fact that dead pneumococcic material does not contain the active poisons found in infection. Hirschfelder² has made an extract of living pneumococci with an alkaline pancreatin solution and reports good results in ten cases. Rosenow has found

¹Wadsworth: J. Exper. M., 1912, XVI, 54, 78.

²Hirschfelder: J. Am. M. Ass., 1912, LIX, 1373.

that antibodies are developed more rapidly on injection of the autolysates of pneumococci after the toxic element has been removed. He reports¹ a definite reduction of mortality in 130 cases of lobar pneumonia treated. Generally it may be accepted that bacterins in pneumonia are of doubtful value and that possibly the results of active immunization with some of the autolysates of pneumococci decrease the mortality. Rose now reports a mortality of 32 per cent. in the treated and 50 per cent. in the untreated cases in the Cook County Hospital in the winter of 1911-12. Whether bacterial vaccines or autolysates are used active immunization should be begun early.

Bacterial vaccines have been of considerable value in the active immunization against pneumococcus infections of the other tissues especially when the infections are chronic. As is the case in all mixed infections which pneumococcus infections are likely to be, mixed vaccines may be necessary.

Serum Therapy and Passive Immunization.—Encouraged by the results of active immunization of animals against pneumococcus infections, attempts have been made to produce passive immunization by the injection of sera from animals artificially immunized. It has been found that to a certain degree serum from actively immunized animals has a protective value but protective action is not necessarily indicative of curative action. Cole prepared animals so that they furnished serum which would protect against 1,000,000 lethal doses if the pneumococci were injected simultaneously with the antiserum but if the pneumococci were injected a few hours before the antiserum there was no protection. Neufeld believes that the amounts of antipneumococcic serum injected are too small. Dochez has however found that after a certain maximum amount of culture is injected no amount of antipneumococcic serum will save the animal and from this he concludes that where infection is severe the body is not able to respond sufficiently no matter how much serum is injected.

In the preparation of antipneumococci serum various points must be remembered. There are many strains of pneumococci

¹Rosenow: J. Am. M. Ass., 1912, LIX, 795.

so that in the active immunization of animals that are to furnish the serum various isolations should be used. Injection of autolysates, especially the toxic portions, does not yield much immunity nor do injections of killed cultures. In most cases immunization of the animals that are to yield the serum is begun by injecting killed cultures. This is followed by injections of autolysates but to make the serum efficient injections of virulent living cultures are necessary. The action of the serum probably depends on lysins and bacteriotropins. The serum is not standardized to any degree of value in treatment. The best known antipneumococcic sera are those of Roemer and Pane.

In treatment of pneumonia with antipneumococcic serum it is to be remembered that infection is severe and that therefore large injections of serum should be made very early in the disease. Injections of at least 20 c. c. should be made twice a day. None of the other known methods of treatment should be suspended during serum treatment.

The results which have been attained by the use of the serum have varied a great deal. Some observers have lauded the use of this serum but when all cases are considered the results in pneumonia are not convincing as far as the curative effects are concerned. The reasons for the failure of a specific antipneumococcic serum in pneumonia are probably dependent on the inability of the body to react sufficiently after the disease is far advanced and the difficulty in determining the species of organism causing the pneumonia. The latter reason must be evident because the disease of lobar pneumonia may be caused by various different species of organisms. Although in general the therapeutic action of antipneumococcic serum has not been marked still physicians who have used it in pneumonia have been impressed with the general improvement of the patient, the lowering of the temperature and the absence of complications. Important untoward symptoms seldom have developed from its use.

While antipneumococcic serum is not definitely a curative measure the physician is warranted in using it whenever the pneumococcus causes severe infection in pneumonia, septi-

cæmia, peritonitis, and so on. In meningitis due to pneumococci, antipneumococcic serum has been used to advantage when injected into the spinal cord. In chronic and local infections specific vaccine therapy is preferable to serum therapy.

LEUCOCYTIC EXTRACT IN THE TREATMENT OF PNEUMONIA.—Hiss and Zinsser and Floyd and Lucas have obtained favorable results in treatment of lobar pneumonia with leucocytic extract (see page 136). Manoukhine¹ and others have called attention to the lowering of the powers of the leucocytes from the third day of pneumonia until the crisis. Manoukhine believes that the leucocytes are killed by the toxins and so set free antibodies which make the pneumococci susceptible to the phagocytes. In the course of the disease there is first a hyperleucocytosis, then leucocytolysis and at the crisis marked phagocytosis. In five fatal cases leucocytolysis was absent. In the treatment of pneumonia Manoukhine takes the leucocytes from seven cubic centimeters of the patient's own blood, kills the leucocytes by freezing, then thaws them out and suspends them in one cubic centimeter of physiological salt solution. Other investigators have injected heated patient's blood or leucocytes, rabbit's blood and even ferments. Injection especially of the killed leucocytes to stimulate leucocytosis, leucocytolysis and phagocytosis opens a new field with encouraging prospects.

Lamar's experiments on the action of soaps, serum and boracic acid have received some attention but have not been tried clinically. Morgenroth's derivative of quinin has been tried clinically but the chemical has produced some undesirable symptoms so that it has fallen into disuse as a therapeutic agent.

Prophylaxis.—Preventive measures against pneumococcus infections are dependent principally on eliminating the predisposing factors to disease. Avoidance of exposure, alcoholic intoxication and so on are of importance. Active and passive immunization are of practical importance apparently.

¹Manoukhine: Arch. d. mal. du coeur, (etc.), 1912, V, 385.

INFECTIONS WITH BACTERIUM PNEUMONIÆ (FRIEDLÄNDER).

This organism was discovered by Friedländer while attempting to establish the etiological factor in croupous pneumonia. Since then it has however been found that it is not the usual cause of croupous or lobar pneumonia. It is rather widely distributed, is occasionally found in the normal mouth but more frequently in the nose. The principal diseases caused in man by this bacillus are pneumonia, rhinitis, otitis media, caries of the bones, and occasionally abscess, peritonitis and endocarditis. The diseases caused may be very serious and even fatal. In some cases it causes a secondary and mixed infection.

Differential Specific Diagnosis.—CLINICAL.—The principal disease is pneumonia. This is not a true lobar pneumonia although it may begin as such but afterwards runs an atypical course, resolution being delayed and the fever prolonged. The lungs themselves are usually quite extensively involved and the exudate is sticky. Apparently the pneumonia caused by this organism occurs at times in epidemic form.

BACTERIOLOGICAL DIAGNOSIS.—The bacteria grow relatively easily on the ordinary culture media. Morphologically *Bact. pneumoniae* is a rather large bacillus, not holding Gram's stain and having a capsule.

Immunity and Specific Therapy.—Relatively little is known about immunity to this organism. Many stock mixed vaccines on the market contain the organism but as has been stated repeatedly the use of a vaccine containing organisms not playing a part in the infection is not advisable.

INFECTION WITH ORGANISM PRODUCING WHOOP- ING COUGH OR PERTUSSIS.

This is a highly contagious disease occurring epidemically and endemically, characterized by catarrhal and nervous symptoms and has as its principal and characteristic symptom a peculiar cough ending in the familiar "whoop." Various micro-organisms have been regarded as the etiological factor,

Of these the influenza-like *Bacillus pertussis* of Spengler, Jochman, Krause and others has received much attention. In 1905 Bordét and Gengou described as the causal organism a short, ovoid, non-motile bacillus not holding Gram's stain. This organism they found in overwhelming numbers and in almost a pure state in the viscid sputum in whooping cough. Because of this and the fact that it gives a complement fixation reaction (see page 59) they regarded it as the etiological factor in the disease. Wollstein¹ found this bacillus present in the sputum early in the cases of pertussis and in the lungs at autopsy. After the second week of the disease it was found with difficulty. Influenza bacilli she found quite constantly throughout the disease. Recently more evidence that the Bordét-Gengou bacillus is the causal organism in whooping cough has been furnished by experimental animal inoculation. Klimenko and Fränkel were able to produce pertussis in monkeys. Recently Inaba² produced typical whooping cough in the ape, the incubation period being thirteen days. After forty-two days of the disease the animal had apparently recovered. Mallory and Horner³ have shown that the primary lesion consists in the presence of masses of the Bordét-Gengou bacillus between the cilia of the epithelial cells lining the trachea and bronchi. The bacilli act principally by sticking together the cilia and thus interfering with their normal movements. Lesions similar to those observed in man Mallory, Horner and Henderson⁴ were able to produce experimentally in animals. This helps to establish more firmly the etiological importance of the Bordét-Gengou bacillus in pertussis. Thus the relation of the Bordét-Gengou bacillus to whooping cough seems well established.

Differential Specific Diagnosis.—The diagnosis of whooping cough is almost always made clinically. The clinical diagnosis is based on the presence of a cough increasing in severity each paroxysm ending in a "whoop" and not accompanied by fever or physical signs. If it is found that the organism of pertussis causes other infections, the fixation of complement

¹Wollstein: J. Exper. M., XI, 1909, 41.

²Inaba: Ztschr. f. Kinderh., 1912, Orig., IV, 254.

³Mallory and Horner: J. Med. Research, 1912, XXVII, 115.

⁴Mallory, Horner and Henderson: J. Med. Research, 1913, XXVII, 391.

test may be necessary. Bacteriological examination in pertussis is beset with difficulties and the diagnosis clinically is so definite that cultures and smears are seldom made.

Immunity and Specific Therapy.—One attack of the disease usually confers immunity. Experimental immunization and its value are still questionable, many of the reports being based on organisms other than the fairly generally accepted etiological factor discovered by Bordét and Gengou.

Vaccine Therapy and Active Immunization.—Ladd encouraged by the experimental evidence of the relation of the Bordét-Gengou bacillus to whooping cough tried active immunization during the disease. Recently a vaccine of this type has been on the market and good results have been reported. From twenty to forty million killed bacilli are injected at intervals of about five days. A few days after the first injection it is claimed that the paroxysms decrease in number and severity and that on the whole the disease is shortened and less severe.

Serum Therapy and Passive Immunization.—This has not advanced markedly. Various investigators have prepared sera. The serum of Manicatide was obtained from animals immunized to what he believed to be the etiological factor. Sera from animals immunized to the Jochman-Krause influenza-like bacillus have been of little value. Bordét's serum has likewise been of doubtful value. On the whole serum therapy in pertussis has made little progress.

Prophylaxis.—The disease is especially contagious. Generally quarantine is enforced for six to eight weeks and certainly until the paroxysms have ceased. Vaccination has apparently been of value and is to be recommended whenever a susceptible child has been exposed to the disease. The disease is so severe and is fatal so frequently that needless exposure so that immunity may be obtained is not warranted.

INFECTIONS WITH BACTERIUM INFLUENZÆ.

Various diseases of the respiratory system are called influenza but not nearly all of them are caused by *Bact. influenza*. While the bacillus of influenza first described by Richard

Pfeiffer in 1892 causes principally a disease of the respiratory tract, it also is the cause of pleuritis, lung abscess, lung gangrene, middle ear disease, conjunctivitis, meningitis, endocarditis, peritonitis, septicæmia, and so on.

Differential Specific Diagnosis.—CLINICAL.—Influenza is a pandemic disease characterized by great rapidity of extension and infection of large numbers of people. Clinical influenza usually begins acutely with a chill followed by fever, prostration and pains in the limbs, head and back. Soon after the onset of fever there is a catarrhal condition of the respiratory tract, coryza, conjunctivitis, laryngitis and bronchitis. There is much expectoration at first tough and mucoid, later purulent. In many cases influenza is followed by pneumonia. Influenzal pneumonia is usually lobular or bronchial and frequently is complicated by a chronic pleuritis. Meningitis due to *Bact. influenza* according to Wollstein¹ is more frequent in infants and children than in adults. It usually follows influenzal disease of the respiratory tract. Whenever pneumonia, meningitis, peritonitis, endocarditis and so on occur during or immediately after an attack of influenza, the new condition is probably also due to *Bact. influenza*.

BACTERIOLOGICAL DIAGNOSIS.—While the organism of influenza has been accurately described there still is much confusion in regard to its recognition and identification. There are a number of organisms resembling *Bact. influenza* morphologically and culturally. By some these have been called pseudo-influenza bacilli. Wollstein² believes that the term "pseudo-influenza" should be discarded because she believes they all belong to the same family although the ability to produce disease apparently varies.

The influenza bacillus is short, very small, and rounded at the ends. It does not hold the Gram stain. It can be identified in stained specimens made directly from the sputum and secretions but by cultural methods a better diagnosis can be made. It only grows in medium containing blood or hæmoglobin, and on agar containing either of these after twenty-four hours of incubation at 37° C. produces very small transparent

¹Wollstein: J. Exper. M., 1911, XIV, 73.

²Wollstein: Jour. Exper. Med., 1906, VIII, 681.

colonies resembling dewdrops. If staphylococci or certain other species are present in the culture *Bact. influenza* grows more rapidly. When stained preparations from cultures are made the bacilli frequently occur in pairs and may be confused with diplococci. Cultures from the circulating blood are of value in the diagnosis. When meningitis exists lumbar puncture and cultures of the spinal fluid should be made. The organisms are usually virulent for white mice and guinea-pigs.

SERUM DIAGNOSIS.—Serum tests offer but little assistance in diagnosis because antibodies develop only slowly. Wollstein seldom got positive agglutination tests in dilutions greater than 1:10.

Immunity and Specific Therapy.—Man is very susceptible to influenza. It has been held by some that one attack predisposes to a second one but this is not generally accepted. Apparently however any immunity that may arise from an attack is of short duration. Experimental immunization in animals is generally regarded as unsuccessful although agglutinins and opsonins are produced.

Vaccine Therapy and Active Immunization.—Little is to be expected from active immunization in acute influenza because the duration of the disease is usually so short that recovery has occurred before the products of actively induced immunization appear. In chronic infections injections of killed cultures of *Bact. influenza* probably are of value. Because other micro-organisms play an important part in influenzal infections, active immunization to these may be of value. There are on the market many mixed vaccines called "mixed influenza," "coryza" and so on vaccines. These all contain some influenza bacilli. Stock vaccines made up of organisms not taking part in the infection are not to be recommended and in every case the etiological factors should be determined before vaccine treatment is begun. In active immunization to *Bact. influenza* the selection of strains used is of importance. Wollstein has shown the difference in immunizing value of various strains, a virulent strain obtained from the respiratory tract producing agglutinins and opsonins to a considerable degree in a goat artificially immunized.

Serum Therapy or Passive Immunization.—Experimentally it has been found that animals can be so immunized that their serum has a protective value in other animals. Curative effects have however been seldom observed. Wollstein immunized a goat by injections of living virulent strains of *Bact. influenzae* and obtained serum having an opsonic value of 1 to 5,000. There were also agglutinins but the bactericidal power was slight and fixation of complement was not positive in dilutions of 1 to 100. By injecting this serum into the subdural space she obtained marked curative effects in influenzal meningitis in the lower species of monkeys. The curative value of the serum apparently lay in the increased phagocytosis and the decrease in rate of multiplication of the bacteria. Whether anti-influenza serum will have the same beneficial effect in other influenza infections and then only when the serum is directly applied is still to be solved.

Prophylaxis.—It is difficult to comply with the requirements that will insure prevention of spread of the disease. Because of the high death rate and frequent cause of fatal complication in the young and old and in those having pulmonary tuberculosis, all should be protected against contracting the disease. Isolation is the only means available to accomplish this, active and passive immunization being of little value.

INFECTIONS WITH DIPLOCOCCUS INTRACELLULARIS (Meningococcus).

Acute meningitis or inflammation of the meninges may be caused by a variety of organisms. Among these the most important ones are *Diplococcus intracellularis* (Meningococcus), *Mic. pneumoniae*, *Bact. tuberculosis*, *Bac. typhosus*, *Strep. pyogenes*, *Bact. influenzae*, *Mic. pyogenes* and so on. Since 1805 an epidemic form of primary meningitis has been recognized and in 1887 Weichselbaum discovered its etiological factor. The other organisms causing meningitis usually do not produce the so-called primary meningitis although *Micrococcus pneumoniae* may. Epidemic meningitis has been endemic in our larger cities for many years and practically every year there occur in different parts of the world extensive epidemics of the disease.

While the disease is referred to as a primary meningitis there is evidence that the initial infection is in the nose and throat and from here travels by the blood stream to the meninges in which the organisms lodge due to an apparent affinity for these tissues. Apparently not every infection even of the meninges leads to development of the disease, a lowering of the resistance of the individual or great virulence of the meningococci being necessary. Furthermore the cerebrospinal fluid shows less bacterial antagonism than does the blood so that the cerebrospinal canal may present the point of lower resistance. The number of carriers of meningococci is far greater than the number of cases of epidemic meningitis. The carriers in some cases have a nasopharyngitis or other local inflammatory condition but in most carriers there are no evidences of disease or discomfort. The disease in some epidemics affects mostly infants while in others chiefly older children or adults are affected.

Differential Specific Diagnosis.—Since the introduction of subdural injections of antimeningitis serum, diagnosis of the etiological factor in the disease has become most important.

CLINICAL.—The symptoms of acute meningitis regardless of the causal micro-organism are such that clinical symptoms and signs alone will not suffice to diagnose the etiological factor. For this lumbar puncture and bacteriological examination of the fluid give the best positive diagnosis. The most valuable early clinical symptoms of acute meningitis are sudden onset of intense headache, vomiting, fever, prostration, opisthotonos and Kernig's sign. Early meningitis may be confused with pneumonia or other diseases with cerebral symptoms. In the cases of acute meningitis it is most valuable to remember that a primary meningitis is usually caused by meningococci. Tuberculous meningitis sometimes offers difficulty in the differential clinical diagnosis because of resemblance of the conditions and because of the failure to diagnose the existing tuberculosis in other parts of the body. The most valuable diagnostic symptoms of tuberculous meningitis are persistent drowsiness, constipation, slight fever, and irregular vomiting, pulse and respiration.

BACTERIOLOGICAL DIAGNOSIS.—Bacteriological examination of the fluid drawn by lumbar puncture gives the best specific diagnosis. For making the lumbar puncture the ordinary surgical exploring needle or Quincke's needle may be used. The patient is usually placed on the right side with the thighs flexed toward the abdomen or else placed in a sitting position with the head flexed forward so as to separate the spines and lamellæ of the vertebræ. After this the patient's back is well cleaned with iodin and alcohol or ether and alcohol. The operator's hands should be cleaned in a similar manner. Usually it is not necessary to use an anæsthetic but when it is necessary, primary ether anæsthesia is sufficient and preferred. The sterile needle is inserted in the median line between the third and fourth lumbar vertebræ which point is on the line with the highest part of the iliac crest. The canal is reached at a depth of about one to one and a half inches. It is easy for the operator to determine when the canal is reached by the slight tick felt when the membrane is punctured. After this the trocar is removed when the spinal fluid should flow freely. If this is not the case the needle should be slightly withdrawn and if still no fluid flows out the canal has probably not been entered, the needle is plugged or else the fluid is too thick to flow through it.

The cerebrospinal fluid in epidemic meningitis except in the very earliest cases is turbid and contains leucocytes and meningococci. When the fluid is clear and no organisms are found the case usually is not one of acute epidemic meningitis. The globulin test of Noguchi and some of the other tests (see page 85) may be of value to determine that a meningitis exists but they are of no value in distinguishing between the different forms. Lucas¹ on examining over five hundred specimens of cerebrospinal fluids found there is no specificity of the cell findings in the spinal fluid in various meningeal inflammations.

For the identification of the meningococcus the fluid is usually centrifugalized in sterile tubes although at times this is not necessary because of the large numbers of pus cells and micro-organisms. From the sediment stained preparations

¹Lucas: *Am. J. Dis. Child.*, 1911, Vol. I, 230.

and cultures are made. In many cases stained preparations are sufficient. Because of the value of specific serum therapy in this disease when administered early, the finding of Gram positive diplococci many of which are intracellular certainly must be regarded as a tentative positive diagnosis. If cultures are to be made the material should be put into agar plates soon after drawing the serum. Early inoculation of the medium is necessary because the cocci die and are dissolved in a short time in drawn spinal fluid. The agar should contain ascitic fluid or probably better, sheep serum. Flexner¹ found that for producing massive cultures Hiss' sheep serum water mixed with beef infusion agar containing two per cent. of glucose is most satisfactory. The colonies are small after 24 hours and grow best when incubated at 37° C. Dunham and Elser have determined the sugar reactions. In dextrose and maltose in Hiss' sheep-serum-water-litmus solution slight acidity is produced though the medium is not coagulated. They do not produce gas in these sugars. Flexner relies much on the fermentation tests in differentiating meningococci from other diplococci. Blood cultures are of little value, not because the organisms do not enter the blood stream but because they do not survive long in the circulating blood. It must always be remembered that many species of bacteria can cause meningitis so that the diagnosis of the etiological factor is essential if specific therapy is to be employed.

SERUM DIAGNOSIS.—The serum diagnosis of infection with *Diplococcus intracellularis* is generally not very satisfactory. In the blood of meningitis patients Meakins found antibodies giving a positive fixation of complement reaction, von Lingelsheim, Kutscher and others obtained positive agglutination reactions and Houston and Rankin and others noted increase in opsonins. Generally however because meningococci are dissolved so rapidly in blood serum and agglutination reactions have been found to be non-specific, serum tests are seldom employed. Positive precipitin, complement fixation and agglutination tests have been obtained with cerebrospinal fluid of epidemic meningitis patients. Collignon and Pilod² have tried

¹Flexner: J. Exper. M., 1907, IX, 105.

²Collignon and Pilod: Presse med., 1911, XIX, 723.

a precipitin reaction developed by Vincent and others. The test is simple: one or two drops of preservative-free antimeningitis serum are added to a tube of freshly drawn cerebrospinal fluid and the mixture is incubated at 52° C. for a few hours. A positive reaction is demonstrated by the formation of a precipitate. The test is delicate and claimed to be of especial value when the cerebrospinal fluid contains only few meningococci. Bacteriological examination of the cerebrospinal fluid is so much more satisfactory than serum and spinal fluid reactions in the diagnosis of epidemic meningitis that bacteriological examinations are to be recommended.

Immunity and Specific Therapy.—Second attacks of acute epidemic meningitis have been rare. Attempts at immunization of animals have resulted in confusion. The meningococcus while producing no extracellular toxin produces a toxic autolysate. In the various experimental immunizations of animals immunity to meningococci and to toxic autolysates have been confused. Jaeger in 1903 found agglutinins in immunized rabbits and Lipierre injected animals with culture and toxin and found them to be immunized and their serum to contain protective and curative properties. Scientific immunization since 1906 has been more dependable for in that year Kolle and Wassermann, Jochman, and Flexner prepared antimeningococcus serum of real curative value.

Vaccine Therapy and Active Immunization.—Active immunization for curative purposes is of little benefit and it was not until injection of killed cultures for prophylactic purposes was tried by Sophian that active immunization to this disease attracted serious consideration in this country. He has found that vaccination with killed cultures of meningococci is immediately followed by fever, leucocytosis and an increase in agglutinins and complement fixation bodies in the circulating blood. Probably the antibodies do not appear in the cerebrospinal fluid for some time but inasmuch as epidemic meningitis is preceded by a local and blood stream infection, protective vaccination gives an immunity. The duration of this immunity Sophian believes to be at least one year. Active immunization probably also is of some value in subacute and chronic cases

of meningitis but in all of these cases passive immunization should first be used.

Serum Therapy and Passive Immunization.—Agglutinating substances were discovered in 1903 by Jaeger in rabbits experimentally immunized to meningococci. Since then numerous attempts have been made to produce specific antisera to be used in passive immunization of the human. It was not until 1906 however that injection of such antimeningococcic serum was followed by any degree of success. In 1906 Kolle and Wassermann reported results on the use of specific meningococcus serum which they had been able to produce in animals either by intravenous or subcutaneous injections of killed and later living cultures of meningococci. Their serum from immunized animals was controlled and tested by determinations of agglutinins and amboceptors giving the complement fixation reaction of Bordét and Gengou. Jochman in this same year reported results on a specific antimeningococcic serum prepared after similar methods. The effects obtained by the use of these sera have however not been as favorable as those obtained by the subdural injection of a serum prepared by Flexner and Jobling.

The method of preparation of the curative meningitis serum of Flexner and Jobling is to inject into the horse first increasing doses of killed meningococci, later increasing doses of living meningococci and finally increasing doses of an extract of meningococci. The extract injected contains the endotoxin liberated by the action of meningococcus autolytic enzyme which as Flexner has found is able to destroy the cell substance of the meningococcus.

The action of the Flexner-Jobling serum is due to bacteriolytic amboceptors which disintegrate the meningococci, opsonins that stimulate phagocytosis and to the anti-endotoxic action on the toxic autolysates of the meningococci. Determination of the strength of the serum is generally unsatisfactory. Jobling believes that the serum should still be opsonifying in dilutions of 1:5000. There undoubtedly is some difference in the potency of the sera on the market as is evidenced by the curative value. It is recommended that the practitioner use

serum prepared by the standard manufacturers and to administer serum prepared in a different laboratory if beneficial results are not obtained on the second subdural injection of the first serum used.

The method of introduction of the Flexner-Jobling serum has probably been responsible to a large degree for the success of serum treatment of epidemic meningitis. The serum should be kept on ice until it is to be administered. Just before injection it should be warmed. Lumbar puncture is made as has already been explained and fluid drawn off. Flexner and Jobling advised injection of the same amount of serum as of cerebrospinal fluid removed and at first recommended injections of not more than 30 c. c. Since the first work much larger doses, even up to 60 c. c., have been injected. Sophian has called attention to the value of controlling the withdrawal of cerebrospinal fluid and the injection of antimeningitis serum by blood pressure determinations. A drop of 10 mm. of mercury while withdrawing cerebrospinal fluid he believes indicates that no more fluid should be withdrawn. Rapid injections of serum cause a rapid drop of the blood pressure and therefore serum injections into the cerebrospinal canal should be made only slowly and stopped whenever there is a drop of blood pressure corresponding to 20 millimeters of mercury. The drop of the blood pressure cannot be reliably obtained from the pulse. It is accompanied by stupor, superficial and irregular respirations, dilatation of the pupils, incontinence of urine and feces, and so on. When these symptoms occur some fluid should at once be withdrawn again and atropin or epinephrin given.

One injection is seldom sufficient. If the case is severe the second injection should be made within twelve hours but in most cases injections are made twenty-four hours apart. These are repeated until improvement occurs both in the symptoms and appearance of the cerebrospinal fluid withdrawn. Probably the safest rule is to repeat injections until the fluid has been free from meningococci for two days because the mere lowering of temperature and decrease of meningeal symptoms may be misleading. When there is recurrence the serum should again be used if meningococci are found in the cerebrospinal

fluid. The total amount of serum injected is of little importance, as much as 240 c. c. having been injected into the patient. When necessary children can be given as large doses as are given to adults. The earlier the injections are made the better. The persistence of *Diplococcus intracellularis* in the lateral ventricles after apparent death of the organism in the spinal subarachnoid space has been emphasized by Knox and Sladen. For treatment of this condition Cushing and Sladen have suggested the advisability of ventricular puncture and injection of antimeningococcus serum.

The results which have been obtained by the use of anti-meningococcus serum as made by Flexner and Jobling have been so beneficial in acute and chronic cases of meningitis due to the *Diplococcus intracellularis* that its use is indicated as a specific therapeutic measure. Before the introduction of the newer sera the death rate was about 70 per cent. while in cases in which they are used the death rate is about 30 per cent. In untreated cases the duration of the disease is five or six weeks and when the serum is used it is about two weeks. Complications in serum-treated cases are quite rare although in children there is sometimes an early temporary deafness. If good results are to be obtained a good diagnosis must be made and again the warning is given that organisms other than *Diplococcus intracellularis* cause meningitis and in such cases anti-meningitis serum is of no therapeutic value.

Prophylaxis.—The prophylactic measures of importance have related principally to quarantine of patients and all attendants. Hall has advised the use of large quantities of water and alkaline diuretics and the abstinence from sugar and syrup to decrease acidity of the urine. Because the organisms occur in the nasal secretions of patients and attendants, antiseptic gargles and nasal sprays are recommended by some and objected to by others. Active immunization for prophylactic purposes promises a good deal and should be resorted to for the present to protect those exposed to the disease although at this time general active immunization of communities hardly seems warranted.

INFECTIONS WITH BACTERIUM TUBERCULOSIS.

The transmissibility of tuberculosis while long suspected was only proven by Klencke in 1843 and the causal organism was not described until 1882. Koch identified the organism which has since been known as *Bacterium tuberculosis*. The disease tuberculosis is not only a human disease but occurs in various animals. While the organisms producing tuberculosis in man and animals resemble each other, still there are certain characteristics by which various types or varieties can be distinguished. The distinct types of tubercle bacilli recognized may be conveniently classified according to their source or origin into human, bovine, avian and those from the cold-blooded animals. Although the types of tubercle bacilli are found chiefly in these animals all of the types from the warm-blooded animals produce disease in certain other animals. Because of this the tuberculosis of most importance to man is that which occurs in man and the animals whose flesh or secretions are used as food. The bacilli may produce disease in almost any part of the body. The form occurring principally in man is tuberculosis of the lungs and is usually caused by the human type of tubercle bacillus. The intestinal form in children is frequently due to the bovine type of the bacillus. In earlier days tuberculosis was supposed to be inherited but now we know that infection even in utero is seldom and that tuberculosis of either or both parents only offers more frequent opportunity for infection and leads to offspring with lowered powers of resistance. The course of tuberculosis in man varies. Schut¹ classifies tuberculosis into three groups: obsolete, latent and manifest. The manifest lesions he divides into exudative and proliferative, each of these into non-progressive and progressive, and the latter into acute and chronic. In the exudative form exudation and cell destruction with sputum and fever prevail. The proliferative type of disease he regards more favorably as to prognosis because of connective tissue formation. To determine the type repeated examinations are necessary.

¹Schut: Wien. klin. Wchnschr., 1912, XXV, 827.

Differential Specific Diagnosis.—Specific diagnosis for clinical purposes is seldom carried to differentiation of the type of tubercle bacillus causing the infection.

CLINICAL.—While there are various symptoms and signs that aid in the diagnosis they are never relied upon entirely. Tubercle bacillus infections are usually accompanied by a low grade of fever characterized by a maximum between two and six P. M. and a minimum between two and six A. M.; during the latter period there is usually perspiration known as a night sweat. Sometimes the fever is not remittent but is intermittent with a daily chill followed by fever and perspiration as in malaria with which early tuberculosis is at time confused. As the disease progresses there is emaciation and anemia. Pulmonary tuberculosis most frequently starts in the upper right lobe. For the diagnosis of phthisis reference is made to the text-books. Dry pleurisy is common in the early stages of tuberculosis. When pneumonia does not go on to complete recovery, tuberculosis must always be suspected. When bones are involved disease of the joints should suggest tuberculosis.

BACTERIOLOGICAL DIAGNOSIS.—The positive diagnosis of tuberculosis can only be made with certainty by the demonstration of the presence of the bacillus of tuberculosis. Owing to the staining peculiarities it is possible by the examination of properly made and stained microscopic preparations to make an almost definite diagnosis of the organism. The difficulty in the diagnosis is not met with in the differentiation of this organism from other bacteria but in obtaining the material in which the tubercle bacillus is present. This last fact is to be accounted for in various ways:

1. Distribution of tubercles in the body; the lesions being found in the lungs, bones, joints, intestines, lymph glands, kidneys, brain, peritoneum, spleen, liver, genito-urinary tract and so on.

2. Distribution of bacilli in lesions; for while they are without doubt present in all tuberculosis lesions yet they are present only in small numbers in some of these especially in the chronic tuberculous processes.

3. Material from the foci may or may not be giving off tubercle bacilli; by some obstruction the material containing the bacilli may

be held back or part of the discharging material only may contain the bacilli and this material may escape examination.

Because of these various reasons it is evident that bacteriological preparations made directly from material supposedly from tuberculous tissues or foci and stained by the methods designed to demonstrate the presence of tubercle bacilli are not always available or applicable. Direct examination for and demonstration of the presence of tubercle bacilli in suspected material by means of the staining reactions is possible usually only in the sputum and exudates from discharging lesions or from material obtained at operation. Tubercle bacilli can however also be demonstrated in urine, feces and so on but in these cases before making and staining slides usually suspension and centrifugalization are necessary.

Diagnosis of Bact. Tuberculosis in Sputum.—As far as the clinician is concerned the examination of sputum for *Bact. tuberculosis* is of most importance. I have referred earlier to the absence of tubercle bacilli in some of the discharges from tuberculous lesions and to the absence of the organisms in some parts of certain discharges containing the tubercle bacillus. A patient with pulmonary tuberculosis may have an occlusion of the bronchus leading to the lesion; his sputum may contain the bacilli at one time and be free from them at another; or the part of the slide examined may not contain the bacilli. If we consider all of these factors in the positive diagnosis of pulmonary tuberculosis from the examination of the sputum, we can readily see that usually the report "tubercle bacilli not present" need not be final. It is far better to state that "tubercle bacilli were not found" than to report that they are not present. The latter implies that taking into consideration the difficulty of finding and demonstrating the organism the bacteriologist desires another specimen of the patient's sputum if the clinician suspects tuberculosis; while the report that the bacilli are not "present" implies that the question is settled and the patient is free from tuberculosis. To avoid this false impression it has been my custom in all cases from which the clinician has sent me a specimen of sputum for examination and in which the presence of the tubercle bacillus could not be demonstrated, to report that the organism could not be demonstrated or was not "found" and that another specimen for examination was desired.

Without doubt clinicians frequently get unsatisfactory reports from the bacteriologist; especially is this true when the tubercle bacilli are not found in the specimen of sputum sent for examination. To get the most satisfactory results from sputum examination requires the combination of technique, a history concerning the case and a frank statement by the bacteriologist as to his findings and opinion. In the examination of sputum for tubercle bacilli everything that can be done to facilitate the same ought to be taken advantage of. There are certain points in the method of collection of sputum and preparation of bacteriological slides which are not always recognized but which if followed will lead to more positive diagnoses of pulmonary tuberculosis, namely:

(a) *Collection of Sputum.*—Preferably the sputum discharged in the morning should be submitted to examination. When however the amount of sputum is small then the entire amount expectorated in the day should be collected. A wide-mouthed glass bottle with a tight-fitting stopper should be used to collect sputum.

(b) *Selection and Staining of Material for Examination.*—If the sputum is homogeneous there can be little or no selection of material; when however this is not the case then the best results are obtained if the sputum is poured upon a flat surface as into a Petri dish and the purulent, cheesy, brittle, blood-stained or gray particles are selected. This material is then spread on at least two slightly heated glass slides, fixed and then stained for five minutes with hot Ziehl's carbol-fuchsin. This is best accomplished by standing a jar containing the carbol-fuchsin solution in a water bath or pan of water kept hot by a gas or alcohol flame. For decolorization a mixture of ninety-seven parts of 95 per cent. alcohol and three parts of concentrated hydrochloric acid is of great service. This mixture does not decolorize the tubercle bacillus but does remove the carbol-fuchsin from smegma and other acid-fast bacilli. Decolorization requires only a few seconds being continued until only the heavy parts of the smear remain red in color. For the counter-stain, Wright's stain consisting of one-half gram each of methylene blue and sodium carbonate in one hundred cubic centimeters of distilled water, has the advantage that if the slide is overstained the methylene blue can be washed out with warm water. Only about one-half minute is required for counter-staining.

(c) *Search for Tubercle Bacilli.*—Examination of the slides must be made with the oil immersion lens. Careful search is facilitated by the use of the mechanical stage. At least two slides should be examined and two hours must at times be spent in the examination. It is best to keep a record of the time spent in the search if no or only a few tubercle bacilli are found.

(d) *Methods Employed When but Few Tubercle Bacilli Occur in the Sputum.*—If the examination of the slides usually made shows no tubercle bacilli further search must be made before it is decided that no tubercle bacilli are present. If the sputum is made fluid by the addition of one part of a 20-30 per cent. sodium hypochlorite solution (antiformin), allowed to stand for 10 to 15 minutes, then centrifuged and from the sediment smears are made and stained, tubercle bacilli can be demonstrated more easily and in greater numbers. It is possible by this method to demonstrate tubercle bacilli in cases classed as probable or doubtful. This enables the physician to enforce treatment when treatment is of most avail. Animal inoculations at times are resorted to to demonstrate the presence of tubercle bacilli in sputum. For this guinea-pigs are injected subcutaneously, preferably with the sediment of sputum treated for one-half to one hour with one part of a 10 per cent. solution of antiformin. The animal should be weighed before and every day after injection. After about fifteen days if tubercle bacilli are present the superficial glands begin to enlarge and usually the animal dies at the end of four to six weeks. At autopsy the typical lesions of tuberculosis will be found in the various organs.

Diagnosis of Bact. Tuberculosis in Exudates and Operative Material.—The demonstration of the presence of tubercle bacilli in exudates and material obtained at operation is made from stained slides, cut sections and by animal inoculation. Stains of the bacteriological slides and cut sections fail at times to bring out the bacilli and the practitioner never ought to be satisfied with negative reports based on such examinations. The importance of animal inoculation cannot be overemphasized, for very often the first positive diagnosis of tuberculosis is made in this way.

Diagnosis of Bact. Tuberculosis in Urine.—The examination of urine for the presence of tubercle bacilli is of importance when the urinary tract is involved. Because of the large amount of urine

and the relatively few tubercle bacilli that may be present, it is necessary to centrifugalize or sediment out the tubercle bacilli. It is further to be remembered that on the genital organs, the folds of the prepuce and clitoris and anywhere where there is an accumulation of the secretions of the skin the smegma bacillus may be present. This organism has morphological and staining characteristics that frequently make differentiation from the tubercle bacillus difficult. To avoid this difficulty in diagnosis the external genitals ought to be well cleaned and the urine taken carefully. Sometimes the bacteriological method of examination fails and for urine examinations animal inoculations are far more important and reliable.

Diagnosis of Bact. Tuberculosis in Feces.—Feces may contain tubercle bacilli. This does not always indicate an intestinal tuberculosis because sometimes tubercle bacilli are present in the feces as a result of swallowing sputum containing these organisms. Direct examination of feces for tubercle bacilli in bacteriological slides is difficult and is applicable only if purulent or blood-stained mucous masses can be obtained to make the slides. Even at times when pus from tuberculous lesions is spread on the slides the bacilli cannot be demonstrated. Animal inoculation, suspension and centrifugalization are of assistance in the positive diagnosis of the organism of tuberculosis in feces.

Diagnosis of Bact. Tuberculosis in the Circulating Blood.—Clinicians and laboratory men have long tried to get better and earlier means of diagnosing incipient tuberculosis. As early as 1868 Villemin obtained successful inoculations of rabbits with blood from tuberculosis patients. Bergeron concluded that the occurrence of tubercle bacilli in the blood in acute tuberculosis is rare. In 1909 Rosenberger¹ reported finding acid-fast bacilli in the circulating blood of tuberculous individuals. Anderson² in examining the blood of 48 cases of human tuberculosis could not demonstrate the presence of tubercle bacilli in the blood by culture, guinea-pig inoculation or stained smears. Rumpf³ believes that tubercle bacilli occur more frequently in the blood than is supposed, but finds that seldom are guinea-pig inoculations successful. Various other

¹Rosenberger: Am. Jour. Med. Sciences, 1909, II, 267.

²Anderson: Bull. Hyg. Laboratory, No. 57.

³Rumpf: Münch. med. Wehnschr., 1912, LIX, 1951.

observers have found tubercle bacilli in stained smears but get only a few positive animal inoculations. It is possible that the organisms demonstrated are not tubercle bacilli, may not be viable or the animals may be protected by the human serum injected with the bacilli. Klemperer believes that the finding of tubercle bacilli in the blood has no prognostic value and that on the whole the result of a positive finding is to be given no more weight than a positive von Pirquet test.

To demonstrate tubercle bacilli in the blood 10 c. c. of blood are drawn as for a blood culture and 1.5 per cent. sodium citrate in normal salt solution is added to prevent clotting. This mixture is centrifuged rapidly for an hour and smears made from the sediment. These should be stained, decolorized and counter-stained as has already been described under sputum examination.

Cultivation of Bact. Tuberculosis.—This is seldom resorted to for simple diagnostic purposes. Cultivation and animal inoculation are the only means for definitely differentiating between the types of bacteria. For cultivation of the organisms animal inoculation or passage through antiformin to get rid of the other bacteria are usually necessary. The animal used for inoculation is the guinea-pig, while for differentiation between the human and bovine types rabbits are injected with small doses. Rabbits are quite insusceptible to the human type of tubercle bacilli while they succumb to the bovine type. Usually four to six weeks are necessary to kill guinea-pigs or rabbits but before this there is loss of weight and glandular enlargement. If earlier diagnosis is necessary 0.5 c. c. of tuberculin should be given, tuberculous animals succumbing to this dose after about fifteen days of infection have elapsed. Cultures and stained preparations are made from the lesions. Dorsett's egg medium or dog serum medium is usually used for the isolation, a large piece of the tubercle being transferred to the medium. Glycerin serum agar is used for subsequent cultures. The cultures must be incubated for several weeks at 37° C. before growth occurs. It usually is of assistance to break the surface of the medium at the time of inoculation. The organisms will grow on glycerin bouillon if the culture floats on the surface. For greater detail the text books on bacteriology are referred to,

Serum Diagnosis.—The serum diagnosis of tuberculosis is rather unpromising. Arloing and Courmont obtained positive agglutination tests in a large percentage of cases of tuberculosis. Agglutinins are however not developed to a high degree and there is much difficulty in getting good suspensions of the bacilli. Koch has used ground-up tubercle bacilli as are found in his T. R. tuberculin (see Tuberculins): he does not use the results for diagnostic purposes but only to learn the extent of protective substances in the serum. The precipitin test has been used but this has been largely to differentiate between the types of tubercle bacilli. The tuberculo-opsonic index has been used to some extent in diagnosis both with heated and unheated serum. Some of the difficulties have been discussed on page 70. On the whole it is of little value in diagnosis although it may be of assistance in active immunization.

Hypersensibility or Allergy in Diagnosis.—Diagnosis of tuberculosis based on the demonstration of tubercle bacilli is often impossible. This is especially true when the lesion in question is located in such a part of the body that no material can be obtained. At times it is not possible to demonstrate the bacilli in material supposedly from the lesion. To overcome these difficulties new diagnostic agents have been introduced.

In 1890 Robert Koch published experiments in which he brought out the fact that the injection of killed, ground-up tubercle bacilli in suspension in water would, in doses not affecting well animals, kill tuberculous ones. Furthermore if the dose of injected material is reduced sufficiently and correctly adjusted this emulsion will prolong life in a tuberculous animal and at times produce recovery from the disease. Koch found that the injected bacilli are not absorbed and from this concluded that the healing substance is a soluble one and one that comes from the body of the tubercle bacilli.

Based on these observations and conclusions Koch grew tubercle bacilli on a 5 per cent. glycerin bouillon for a period of from six to eight weeks, then boiled the bouillon culture, filtered off the bacilli and evaporated the filtered bouillon down to one-tenth of its original volume, thus obtaining a 50 per cent. glycerin preparation. This has been called "Koch's old tuberculin." Tuberculous guinea-pigs are affected much more by this tuberculin than are well pigs, one-half cubic centimeter causing death in tuberculous animals.

When the amounts injected are correctly graduated it produces beneficial results.

The results of tuberculin treatment are not as satisfactory as had been expected and soon the use of tuberculin as a curative measure dropped to the background. This was not so however in its application as a diagnostic agent. The difference in effect produced by the injection of living and dead tubercle bacilli and of tuberculin in well and tuberculous guinea-pigs gave evidence of increased susceptibility to these substances in tuberculosis. On this has been based the tuberculin method of diagnosis of tuberculosis in man and cattle.

A non-tuberculous guinea-pig will tolerate as much as two cubic centimeters of the concentrated old tuberculin. A tuberculous pig will die when 0.5 c. c. are injected subcutaneously. Man however is much more susceptible to tuberculin. A healthy individual will tolerate 10 milligrams and even at times 50 to 100 milligrams will produce no ill effects, but in a person with tuberculosis doses varying from one-thousandth to ten milligrams cause focal and general reactions of varying degree.

The focal reactions in man can best be seen in lupus and tuberculosis of the joints. From four to six hours after the injection of tuberculin the diseased tissues begin to swell, become red and hot. In the guinea-pig killed during the time of the reaction one observes a marked congestion and hyperæmia about the lesions. In the peritoneum the reaction is nicely demonstrated when there is a tuberculous peritonitis. The reaction may be so severe as to cause necrosis, producing a tendency to demarcation and sloughing off of the tuberculous tissue. In the human this reaction of the tissues is evidenced in tuberculosis of the lungs by increased cough, rales, sputum and accelerated respiration. The action of tuberculin then is one on the tissues containing the tubercle bacilli rather than on the bacilli themselves.

The general reaction in man is usually evidenced by weakness, pains in the joints, chills with subsequent fever and sometimes vomiting. The reaction may be severe making the individual quite ill. In the tuberculous guinea-pig the tuberculin injection is followed by sluggishness and death. The rise in temperature is not much relied on in guinea-pig reactions.

Both the focal and general reactions depend on two factors—

susceptibility and the amount of tuberculin given. Of these two factors the degree of hypersensitization is entirely unknown and is what is being tested for. The amount of tuberculin used in the test however can be regulated. The determination of the quantity of tuberculin injected is of great importance inasmuch as the reactions and disturbances at times are so severe that tuberculin has been given credit for causing new foci of tuberculosis. To avoid these severe reactions small doses of tuberculin are used at first, then gradually the dose is increased, the principle of the best methods now known being to give the least amount that will give definite information as to the presence or absence of tuberculosis.

Hageman¹ has used intracutaneous injection of suspected tuberculous material into tuberculous guinea-pigs for diagnosis of surgical tuberculosis. If the material is tuberculous there is swelling under the skin of the guinea-pig at the site of the inoculation. The swelling has a bluish-red center which is surrounded by an area of inflammation.

THE SUBCUTANEOUS TUBERCULIN TEST.—The methods of conducting tuberculin tests for diagnostic purposes vary to some extent. There are however some general rules that are quite universally followed. Before the test the subjective symptoms as cough, amount of expectoration, presence or absence of pain, mobility and so on are observed. Of all observations temperature is most important. Usually rectal temperatures are taken every two hours for two days before the injection. If there is much fever the patient should be put to bed until the temperature is fairly normal, that is 99° F. or below. Just before injection the patient should be examined carefully and the results of examination carefully noted. Tuberculin is usually injected early in the morning or late in the evening as the reaction comes on in six to twelve hours. The amount of tuberculin injected depends in some degree on the objects of the test which may be to establish that the patient is hypersensitive as is determined from the general reaction or to produce a focal reaction as is determined from examination at the seat of the lesion or lesions. For the former a small dose suffices while to produce clear-cut focal reactions larger doses are necessary. The most satisfactory dosage for adults for the first injection is

¹Hageman: *Beit. z. kl. Chirurgie*, 1912, LXXXII, 1.

one-fifth milligram; if there is no reaction after forty-eight hours then one milligram is injected and if still no reaction occurs then five milligrams are injected. Children receive smaller doses, one-tenth milligram initial dose and one milligram for the final. If there is incomplete evidence of a reaction the same dose is repeated. Koch's old tuberculin (see page 191) is used in the test. Hypodermic subcutaneous injections are usually made in the abdomen or on the back.

The symptoms and signs of a positive reaction vary. They appear in from six to twelve hours and are over with usually in thirty-six hours. The most important symptom is fever which in the mild reactions only reaches 100° F. and in severe ones exceeds 102° F. During the reaction frequently patients have the symptoms observed in influenza, are depressed and have aches in the various parts of the body. There is usually general malaise. At the site of injection there is usually redness and swelling (local reaction). The regional glands are frequently swollen and tender. At the seat of infection there is an inflammatory reaction which is visible if the lesion is situated externally as at the knee and if it is in the lungs there is increased sputum and rales (focal reaction).

The severity of positive reactions is not proportionate to the amount of infection, very slight infections often giving marked reactions. The tuberculin test then is not a diagnostic measure of the disease tuberculosis but furnishes an index of infection with the tubercle bacillus. Too much must not be expected of it and in every case all of the other means of diagnosis must be applied and given consideration. Where there is a positive reaction it is well to keep the patient under observation and to so outline the conditions of life that tuberculous infection will not become tuberculous disease.

THE CUTANEOUS TEST OF VON PIRQUET.—The methods of Von Pirquet were first published in the spring of 1907. He found if he placed a drop of Koch's old tuberculin on the skin of a tuberculous individual and then scarified the skin and allowed the tuberculin to enter the tissues, that at this point a bright red, later dark red, papule would appear. The reaction at times lasted as long as eight days and came out best in cases of skin and gland tuberculosis especially in children under one year of age. Von Pirquet con-

cluded from his investigation that the test is positive for the diagnosis of tuberculosis.

Engel and Bauer from investigations on nursing children obtained at times a positive skin reaction when at autopsy no signs of tuberculosis could be found. The reaction was sometimes very severe and gave the best results in children between the ages of three and fourteen. Wolff-Eisner and Teichman have made efforts to differentiate between the different stages of tuberculosis by the appearance and degree of the skin reaction. The test has been tried on animals especially on cattle, horses and guinea-pigs by Vallee who believes the reaction to be of diagnostic value in tuberculosis.

To make the test the skin of the forearm is well cleaned and dried. After this drops of 50 per cent. sterile glycerin are placed on the skin in two places about four inches apart. Midway between these two a drop of sterile Koch's old tuberculin is placed. Then with a sterile Von Pirquet's borer or a blood sticker or needle slight abrasions of the epidermis are made, first where the glycerin has been placed and finally in the location of the tuberculin. There should be no bleeding and the glycerin and tuberculin should be allowed to infiltrate for about fifteen minutes. The tests are examined at the end of twenty-four hours. Different reactions are observed: (a) a negative reaction in which there is no difference between the tuberculin and control areas; (b) a slight reaction in which there is more redness and infiltration about the tuberculin than about the control areas; (c) a positive reaction in which there is elevation and infiltration about the tuberculin area; (d) a marked reaction in which the process goes on to vesicle formation.

Morland has advocated the quantitative cutaneous tuberculin test of Ellermann and Erlandsen in which various dilutions of tuberculin are used and the reaction area is carefully measured. To this there are many drawbacks. It must be remembered that tuberculin tests only indicate the presence of tubercle bacilli and not tuberculous disease. It is the general consensus of opinion that the test is of value only in the early years of life and that the absence of a positive reaction excludes an active tuberculous process. Because of the simplicity of the test it may then be used in all cases where tuberculosis is suspected, but if there is a positive reaction in a patient more than three years old it should be followed by the sub-

cutaneous test and all the known methods of positive diagnosis. The test is of great value if the tuberculin can be applied directly to the lesions of the skin when a focal cutaneous reaction may occur.

THE PERCUTANEOUS TEST OF MORO AND DOGANOFF.—These men showed that if tuberculin is rubbed into the skin of tuberculous patients there follows a specific reaction. The technique advocated by Moro¹ is to prepare an ointment of equal parts of Koch's old tuberculin and lanolin and of this mixture to rub about one-tenth gram thoroughly into the skin about the nipple, covering an area of about 5 centimeters. A positive reaction appears after twenty-four to forty-eight hours in the form of redness and elevated papules of various sizes.

The results obtained have varied with the different observers. On the whole not all tuberculosis cases respond and the test is not held to be of as much value as the Von Pirquet and eye tests. It probably only should be used when there is objection on the part of the patient and relatives to the subcutaneous, cutaneous and eye tests.

INTRACUTANEOUS TUBERCULIN TEST.—This was proposed by Mendel. It has been found to have some diagnostic value if injections of varying amounts of Koch's old tuberculin are made into the skin of the same arm. Only small amounts of the properly diluted tuberculin are injected, one-twentieth of a cubic centimeter producing in the skin the desired white elevation. Hamman and Wolman² have injected one-twentieth c. c. of pure salt solution as a control; for the tests they injected one-twentieth c. c. each of a 1 to 1,000,000, 1 to 100,000 and 1 to 10,000 dilutions of old tuberculin. If none of the areas reacted less diluted tuberculin was injected. The reaction is about the same as for the cutaneous test and has advantages over the test of Von Pirquet.

OCULAR OR CONJUNCTIVAL TUBERCULIN TEST OF CALMETTE.—Wolff-Eisner in 1907 stated that he had obtained specific reactions on the instillation of tuberculin into the conjunctival sac. Calmette entirely independent of Wolff-Eisner soon after reported a more refined method of obtaining the conjunctival reaction.

In 1891 Koch in giving further information concerning his tuber-

¹Moro: Münch. med. Wchnschr., 1908, LV, 216.

²Hamman and Wolman: Tuberculin in Diagnosis and Treatment, 1912.

culin stated that the active part is a substance that is insoluble in absolute alcohol and that it probably is a derivative of albumin but not a tox-albumin inasmuch as it can be heated to the boiling point and passes rapidly through the membrane of the dialyzer. Inasmuch as glycerin of which the old tuberculin contains 50 per cent. causes irritation of the conjunctiva, Calmette prepared a glycerine-free tuberculin by precipitating out with alcohol the active principle of tuberculin. The alcohol precipitate is soluble in water. Calmette observed that when several drops of a 1 per cent. aqueous solution are placed into the conjunctival sac of the eye of a tuberculous patient there follows several hours after the instillation a marked congestion of the palpebral conjunctiva, a swelling and redness of the caruncle and a fibrinous discharge. This reaction reaches its maximum about ten hours after instillation and disappears in from 18 to 36 hours.

This means of diagnosis of tuberculosis at once attracted much attention and now reports have come from many clinics concerning its value. Some of the reports favor this method for the diagnosis of tuberculosis, while in some clinics unpleasant complications have been observed. The complications in some cases have persisted for some time and have been observed principally in eyes that were defective before the instillation of the tuberculin. At the present time there certainly are some objections to the clinical application of the test. The two chief objections are dependent on its limitations as a means of diagnosis and the complications that may arise in the eye as a result of the test.

The test should never be used when there is disease of the eyes or lids or in the eyes of the aged. The solution should not be stronger than 1 per cent. The method has been tried extensively, the results vary, there are no especial advantages to the method and at times severe untoward reactions occur.

VALUE OF THE VARIOUS TUBERCULIN TESTS.—Not all of the tests give similar results and all of the methods have some shortcomings. Hypersensitiveness to tuberculin unfortunately gives no indication of tuberculous disease but only evidence of the presence of tubercle bacilli in the body. All of the cells and tissues of the body acquire the property, although it may be to a different degree. Severe reactions in any of the tests do not indicate severe infection,

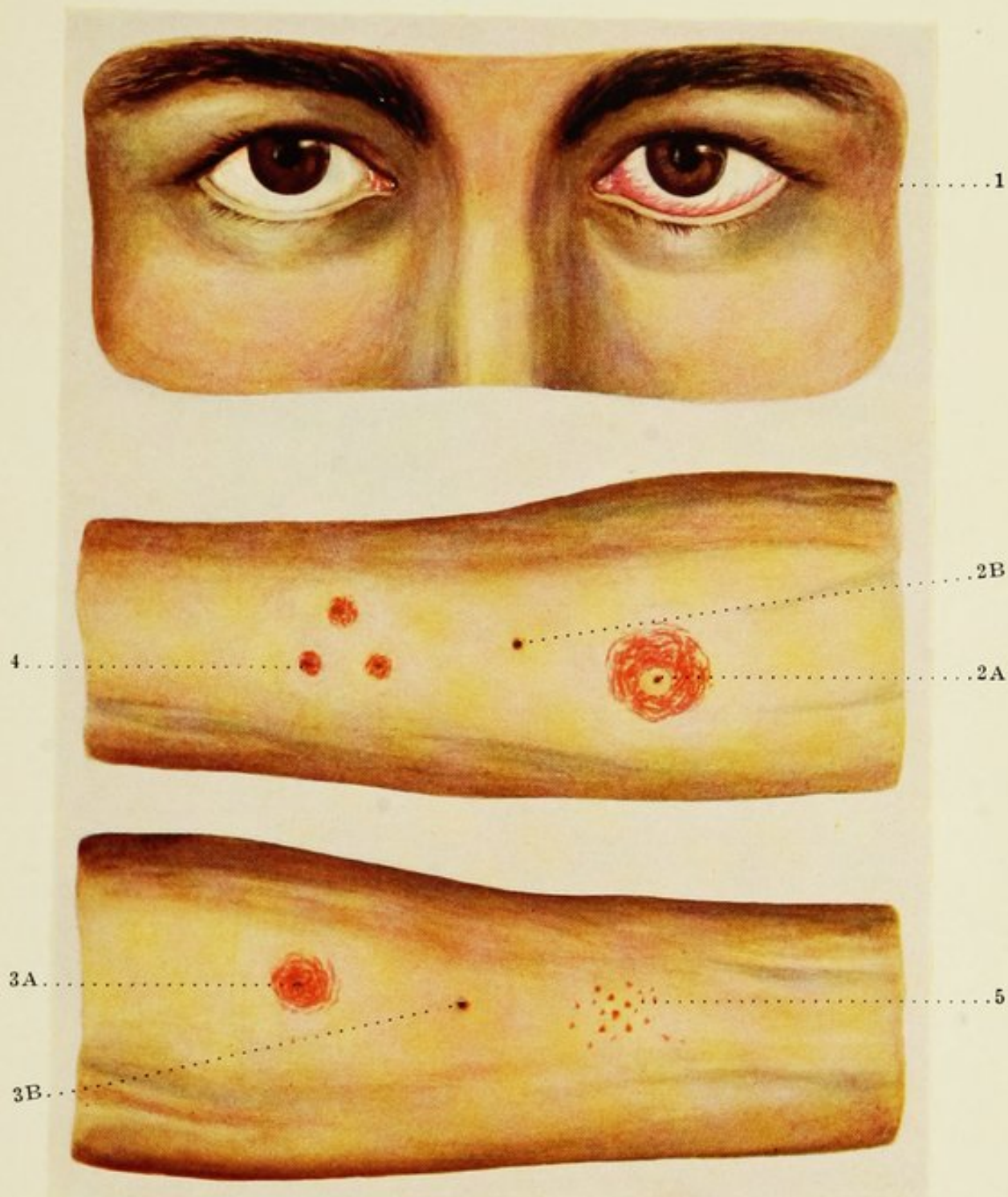


PLATE SHOWING CONJUNCTIVAL, VON PIRQUET, AND MORO REACTIONS. (FROM POTTENGER: TUBERCULIN IN DIAGNOSIS AND TREATMENT.)

1. Conjunctival reaction of left eye.
- 2A. Von Pirquet reaction, well marked; B, control site.
- 3A. Von Pirquet reaction, slight; B, control site.
4. Moro reaction, well marked.
5. Moro reaction, slight.



Of all the tests the intracutaneous one is the most sensitive and the conjunctival the least so. The subcutaneous test is of most assistance clinically. It is necessary that the result of the tuberculin reaction be regarded as furnishing evidence on one of the cardinal symptoms and in itself is of only that importance.

Immunity and Specific Therapy.—Immunization as has been stated earlier may be for protective or curative purposes and may follow a natural or modified course of the disease. Before the time of scientific artificial immunization it was definitely believed that tuberculosis confers no appreciable protection against future development of the disease. In man the question of acquired immunity to tuberculosis is answered usually in the negative. Scrofula and the other forms of local tuberculosis in early life do not produce an immunity to later pulmonary tuberculosis. There however apparently is some increased refractory condition in persons who have once had a tuberculous infection. The refractory condition or immunity to tuberculosis manifests itself principally or almost solely in protecting other parts of the body, but does not hinder the progression of the original lesions. Trustworthy observations made at autopsy on man show that 90 per cent. of adults dying from all causes have at some period of their lives had tuberculous infection in some part of the body while only 15 per cent. of deaths are due to the disease tuberculosis. Of the persons having had tuberculous infections it must be acknowledged that most of them retain in their body viable tubercle bacilli and that cure of tuberculosis need not be due to immunity but rather to effective encapsulation of the bacilli.

Koch's investigations which proved there is a marked difference in the reaction of tuberculous and well animals and man to cutaneous and subcutaneous inoculations of tubercle bacilli and to injections of tuberculin, speak clearly for an immunity to tuberculosis. This altered power of reaction has already been discussed (page 82. Hypersensitiveness to tubercle bacilli and tuberculin agrees in most points with serum hypersensibility or anaphylaxis but differs in these essentials: tuberculin injected into well animals produces no hypersensitiveness and hypersensitiveness cannot be induced passively by injections of serum from sensitized animals into normal animals. Anaphylaxis is regarded as a part of immunization and

therefore tuberculin hypersensitiveness has also usually been looked upon as a part of immunization to tuberculosis.

Vaccine Therapy and Active Immunization.—Active immunization to tuberculosis has been attempted principally along two lines: by the injection of tuberculin and by injection of tubercle bacilli either nonvirulent or killed. Earlier it has been stated that there are different varieties of tubercle bacilli. Among the animals there are varying degrees of susceptibility to these different strains. In man tuberculosis is caused principally by the human type of bacillus although the bovine type causes some of the infections. Guinea-pigs are very susceptible, rabbits much less so and pigeons are immune to the human strain. The bovine type of bacillus is responsible for the infection in most of the mammalia. Guinea-pigs and rabbits are very susceptible to this strain. The avian type of bacillus is responsible for most fowl tuberculosis. Some of the mammalia are quite susceptible to the avian bacillus, rabbits succumb readily although no tubercles and caseation are produced but guinea-pigs are quite immune. The tubercle bacilli of cold-blooded animals produce tuberculosis in fish, turtles and so on but cause no disease in the warm-blooded animals. On the basis of absolute or at least relative immunity of man to avian bacilli and to those causing tuberculosis of cold-blooded animals as well as to old attenuated cultures of the human type, attempts have been made to produce immunity by injection of cultures of these organisms. Courmont and Dor, and Martin and Grancher tried to produce immunity in this way as early as 1890.

To the work of Trudeau and deSchweinitz however belongs the credit of the first successful immunization of animals. Since then these methods have been much used, been modified and found to be of value especially in protecting animals against tuberculosis. It is not possible here to give all of the attempts of this method of immunization and the results obtained. The work of Behring, Neufeld, McFayden, and Pearson and Gilliland must however be mentioned because of the results they have obtained in the immunization of cattle against tuberculosis and the great scientific importance of such successful immunization. Generally it may be said that animals treated with attenuated living human tubercle bacilli

possess a higher grade of immunity than do animals injected with killed bacilli or bacilli of the avian and fish strains.

The use of tuberculin in immunization against tuberculosis is founded on observations originally made by Koch. He observed that while large doses of tubercle bacilli cause death in tuberculous guinea-pigs, small doses prolong life and may even lead to the cure of tuberculosis in these animals. He further observed that the bacilli injected for curative purposes are not absorbed and therefore ascribed the curative action to a soluble substance which he called tuberculin. This he believed to be a soluble toxin responsible for the constitutional symptoms which occur in tuberculous individuals when they receive tuberculin injections. With the development of the side-chain theory of immunity it became the belief that a part of the curative action of tuberculin is due to antibodies formed as a result of stimulation of the cells by tuberculin. With the acceptance of the belief that bacteriolytic substances are only formed after injection of bacteria, tuberculins containing whole or fragmented tubercle bacilli were prepared.

Tuberculins.—The principal tuberculins now used and those on the market belong to different groups:

I. *Preparations Derived from the Culture Fluids.*—Of this group Koch's old tuberculin and Deny's bouillon filtrate are the principal ones.

(a) *Koch's old tuberculin* ("O. T."). This is made by evaporating a six to eight weeks' bouillon culture of tubercle bacilli at the boiling point to one-tenth of its volume and filtering this through a Berkefeld or Chamberland filter to make it germ free.

(b) *Deny's bouillon filtrate* ("B. F."). This is the bouillon on which human type of tubercle bacilli have grown for six to eight weeks, rendered germ free by filtration through a Berkefeld or Chamberland filter. It differs from "O. T." in that it is not heated or concentrated.

(c) Jochman, Jessen and others have made modifications of this group of tuberculins but they are little used.

II. *Preparations of Tubercle Bacilli, Dead or Alive (Vaccines).*—Of these the best known are Koch's bacillus emulsion and Behring's vaccines.

Koch's bacillus emulsion ("B. E."). Tubercle bacilli are grown on bouillon for six to eight weeks, filtered off and ground up. Of the pulverized material one part is emulsified in 100 parts of distilled water. The emulsion is then diluted with an equal part of glycerin. Thus one cubic centimeter of the emulsion contains 5 milligrams of the fragmented bacilli in addition to certain extractives. The bacilli are not heated or washed.

III. *Preparations of Tubercle Bacillus Extractives.*—This group includes extractives with and without attempts at isolation of principles and pure constituents.

Koch's tuberculin residue ("T. R." or New Tuberculin). This is prepared from four to six weeks' bouillon cultures of virulent tubercle bacilli which are filtered off and dried in a vacuum without heat. One gram of the dried bacilli is ground in an agate mortar until all the bacilli are broken up. To the powdered mass 100 c. c. of distilled water is added and then centrifugalized. The supernatant fluid contains extractives, fatty substances and few bacilli—this is known as "tuberkulin oberes" or "T. O." The sediment is again dried, ground up and mixed with a small amount of water. The supernatant fluid after centrifugalization is drawn off and preserved. This process is repeated until almost no sediment remains. All of the supernatant fluid except that first drawn off (T. O.) is mixed and made up to a total of 100 c. c. To this fluid 20 per cent. of glycerin and a little formaldehyde are added. Each cubic centimeter represents about 2 milligrams of solids of the residue from 10 milligrams of tubercle bacilli.

Landmann's tuberculol and Kleb's tuberculocidin represent efforts at obtaining chemical entities from tubercle bacilli. They are little used.

IV. *Tuberculins Made from Tubercle Bacilli Other Than the Human Type, and from Other Acid-Fast Bacilli.*—The best known of these are those made by Spengler, Roux and Calmette.

In the different tuberculins, whether they be derived from the fluid element of the culture or from the bodies of the tubercle bacilli, apparently the same specific substance exists. By chemical means the specific substances in the bacilli and in the tuberculins have not been identified. Any preparation producing the reaction produced by tuberculin in a tuberculous individual or animal is called a tuberculin and the test for it is not a chemical but a biological one.

Before deciding on which one of the different tuberculins is to be used for curative purposes it is necessary to determine what is to be accomplished to effect a cure. Koch's original idea was, inasmuch as tubercle bacilli are found only plentifully in freshly diseased tissues and are scarce in old foci having undergone coagulation necrosis as a result of the harmful substances produced by the tubercle bacilli, that tuberculin producing such coagulation necrosis exerts its curative action by producing conditions which make the opportunity of finding nourishment more difficult for the existence of the tubercle bacilli. Later work makes the acceptance of Koch's necrotization idea as responsible for the healing of tuberculosis impossible. It now is generally accepted that the prolonged effect of administration of small doses of tuberculin results in a

hyperaemia and inflammation which cause either resorption of tuberculous tissue or fibrosis around it.

The character of the healing with and without tuberculin treatment is the same, but apparently fibrosis is more marked and calcification occurs less frequently when tuberculin is properly given. When the tuberculous lesions are in parts accessible to observation there is certainly evidence of healing if tuberculin is correctly given. When there is pulmonary tuberculosis the physical signs change only slowly. On treatment with tuberculin specific agglutinins are undoubtedly produced. Opsonins probably are also produced but our methods of determining the opsonic index are so unreliable that definite conclusions on the increase in opsonins are not warranted. Antituberculin has been found in the serum of some patients receiving tuberculin especially where the tolerance is not increased. After all no matter what the pathological anatomy, symptoms and signs or presence or absence of antibodies may be, the real value of tuberculin rests on the data concerning the years of life added to tuberculous patients receiving tuberculin treatment. The majority of clinicians using tuberculin in the treatment of tuberculosis feel it is a definitely useful agent.

ADMINISTRATION OF TUBERCULIN.—The selection of the kind of tuberculin to be administered has been regarded as of considerable importance. The conception of the immunization accomplished by tuberculin injections differs, there being two important theories. Koch, Wright and others believe that antibody specific to the action of the tubercle bacillus is produced. This according to their views is accomplished through stimulation of all the defenses of the body. In conformity to their views on the objects of tuberculin immunization, tubercle bacilli are injected in the form of "T. R." or "B. E." Maragliano, Sahli, Deny, Trudeau and others adhere to the toxin immunization theory according to which tuberculin injections lead only to tolerance or immunization to the toxin liberated by the tubercle bacillus. In accordance to this theory old tuberculin as well as other tuberculins are injected, the doses being gradually increased to the highest point of tolerance. The lesion according to this theory is only secondarily affected there being no acquisition of immunity to the tubercle bacillus.

Whatever our conception on this may be, the fact must not be

lost sight of that most tuberculous patients obtain simultaneously with improvement under tuberculin treatment an increased tolerance for tuberculin. The various tuberculins proposed and used may be divided into two classes according to the ultimate effects they produce: those that are efficient and those that are not. Of the efficient ones Koch's "O. T.," "T. R." and "B. E." and Deny's "B. F." are the ones most frequently used. They all produce good results in all types and forms of tuberculosis, immunize against each other and all produce the specific reaction. Hamman and Wollman prefer the more soluble forms over the suspensions. The latter produce more local trouble because of irregular absorption and cumulative effects. They use "B. F." largely while Brown prefers "B. E." because it stimulates production of bacteriolysins as well as antitoxins. Hamman and Wollman believe that tuberculins cannot be classified on such grounds, the immunity obtained by all being of the same kind.

The *dosage* and *interval between injections* vary in the different clinics. Koch on the basis of wanting to get the focal necrosis with the scarring and healing obtained in lupus gave large doses and increased them to get marked reactions. It was soon however realized that it is desirable to avoid too severe reactions and that if sufficient tuberculin is given to get evident focal reactions the dose may be so large as to exceed beneficial reactions. Tuberculin now is given more cautiously and while there is still some difference of opinion as to the reaction desired it is now fairly well established that at most only slight constitutional symptoms, as fever, should be produced. The whole idea of present-day tuberculin treatment is to produce no harm.

To accomplish only mild reaction no definite scheme can be advocated, each patient requiring separate study. We must however have an initial dose and this must be so small that no individual is harmed. This can only be based on empirical data. The doses commonly given vary. Adults not in good condition or having fever and children should not receive as large doses as adults in good condition and without fever. Safe doses for the initial injection according to Hamman and Wolman¹ are:

¹Hamman and Wolman: Tuberculin in Diagnosis and Treatment.

TUBERCULIN	INITIAL DOSE.
O. T.	0.000,000,1 to 0.000,001 c. c.
T. R.	0.000,001, to 0.000,1 c. c.
B. E.	0.000,001, to 0.000,1 c. c.
B. F.	0.000,000,01 to 0.000,1 c. c.
Beraneck's	of A/32,0.05 c. c

From the reaction or failure of reaction when these doses are given further doses must be determined. As has already been stated it is desirable to give the largest dose that does not produce a reaction. The signs of reaction manifest themselves at the point of injection by pain, tenderness and swelling; at the seat of infection by congestion and in pulmonary tuberculosis by increased cough, expectoration, dyspnoea and general increase in physical signs; and by the constitutional symptoms of fever, loss of appetite and weight, nausea, vomiting, headache, chills and general depression. Of all the signs, fever, loss of weight and depression are the most important and can be most accurately determined. This does not signify that these are the only possibilities of a reaction that must be looked for. A small rise in temperature alone does not contraindicate increase in dose but when there is slight increase in temperature other manifestations of reaction must be most closely watched for. It must be remembered that even tuberculous patients with normal temperature and not being treated with tuberculin at times have some fever and in all such cases tonsillitis, grip, and so on must be looked for to avoid confusion. Fever due to tuberculin usually comes on within forty-eight hours after injection and is preceded by reaction at the point of the tuberculin injection. The local reaction may manifest itself only as a thickening of the skin or the skin may be red and inflamed. If a local reaction appears it usually follows that a subsequent larger dose will elicit constitutional reactions, while if a smaller dose is injected such injection probably will not be followed by another local reaction.

When it has been determined that there is no reaction from the initial injection of tuberculin, a second injection should be made in three to five days. The dose to be injected again is a matter of judgment. It is desirable to increase the tuberculin as rapidly as

possible, to avoid manifest reactions and still to be sure that the doses are increased as much as is permissible. To do this slight warning signals in the form of local reactions must be obtained from time to time. If there is no slight local reaction there also is no focal reaction, and while it is undesirable to produce any extensive focal reaction a slight focal reaction must be obtained if the patient is to be benefited. The dosage for the first few injections should be increased only slightly to definitely test out the patient's sensibility. If no reaction is obtained from these, larger doses should be given. As has already been stated it is impossible to establish a routine and at best only suggestions are permissible. The best method of avoiding mistakes in injections and records of dosage is to make up a number of dilutions, the following being suggested:

No. 1	of which 1 c. c. contains	1 c. c. of tuberculin.
No. 2	" " " "	0.1 c. c. of tuberculin.
No. 3	" " " "	0.01 c. c. of tuberculin.
No. 4	" " " "	0.001 c. c. of tuberculin.
No. 5	" " " "	0.000,1 c. c. of tuberculin.
No. 6	" " " "	0.000,01 c. c. of tuberculin.
No. 7	" " " "	0.000,001 c. c. of tuberculin.
No. 8	" " " "	0.000,000,1 c. c. of tuberculin.

Dilutions are usually made with physiological salt solution. They should be well shaken before injections are made.

The amounts of these dilutions injected vary from 0.1 to 0.9 c. c. At the first injection usually 0.1 c. c. of No. 7 bottle is injected. If there is no reaction the second injection should usually be 0.2 c. c. and if there is no reaction the subsequent doses are increased 0.2 c. c. at each time until a slight local reaction is obtained. Then it is well to repeat the same amount at the next time. After this if there is no reaction the amount is again increased by 0.2 c. c. of the same dilution. As soon as 0.8 or 0.9 c. c. of a dilution is tolerated, 0.1 c. c. of the next lowest dilution is given. The interval between injections should be from three to five days but if there is much reaction the interval should be lengthened to one or two weeks and the dose actually decreased. It may be well to repeat that there is no routine for all patients, that each patient is a particular object of study and that the patient himself is the best regulator. If a patient is hypersensitive repeated injections of a slightly less amount than produces a reaction usually overcome hypersensitiveness, after which the doses can again be increased.

The site of injection and manner of injection of tuberculin are of some importance. It is advisable not to inject tuberculin deep into the muscles. Injection should be made only under the skin with the opening of the needle facing the skin so that the local reaction, valuable in determining dosage and so on, comes out clearly. The site of injection preferably should not be on the arms or legs as here the reactions are at times severe. The back or abdomen are preferred. The best time for injection is apparently late in the afternoon because then the injection can be omitted if there is the evening rise in temperature. After injection it is usually best to have the patient rest for a time although it is permissible for him to be up and do light work.

The length of time for which tuberculin injections should be continued varies for each patient. In some clinics patients are given tuberculin indefinitely while in others there are periods of rest. Both have advantages and the course to be followed depends on the individual. As control for the continuation of treatment Wright has advocated his opsonic index method; others have used tuberculin as long as and whenever there is a positive cutaneous reaction. In some cases it is easy to tell when recovery from tuberculosis has occurred; in other cases after the maximum dose is reached it should either be maintained for some time or a rest be given to the patient after which the dose should again be brought to the maximum. The maximum dose is empirical to some extent, being 1. c. c. of No. 1 of "B. F." and "O. T." and 2 c. c. of No. 1 of "T. R." and "B. E."

There are various methods of administering tuberculin other than by subcutaneous injection. Of these the oral, rectal and inhalation methods are inaccurate and not so well controlled. The same holds true of applying tuberculin to the focus of the disease. Intravenous injection is attended by some danger.

There has been much consideration of the fitness of patients for tuberculin treatment. The only harm that can be done by careful tuberculin therapy exists when there are complicating diseases. Of these there are few of importance. If the complication is severe little good will result because the system cannot respond properly. Some cases of tuberculosis are however better suited to tuberculin treatment than others. In the first place treatment early in the

disease offers the best prognosis. Tuberculin does not furnish anti-toxin or bactericidal substances—it merely stimulates the natural defenses of the body. It is not to be given more importance than an aid in recovery and cure, ranking in this way with rest, good food and fresh air.

OTHER METHODS OF ACTIVE IMMUNIZATION.—Active immunization of patients by means other than by tuberculin injections are numerous. Some of these have been for curative purposes others for prevention against infection. Attempts at preventive immunization have been made largely in cattle. When tuberculin has not been used for this purpose killed and living virulent and nonvirulent cultures have been injected. Generally when living cultures are injected the variety of tubercle bacillus selected has been one that does not produce disease in the species of animal to be immunized. Injections of living or dead tubercle bacilli usually produce local nodules. All of these methods except those advocated by Behring, Pearson and Gilliland and others for cattle immunization have been found to have no value over tuberculin treatment. Recently Friedman reported a new remedy before the Berlin Medical Society. While he has given little information in regard to his preparation it is supposedly founded on immunization as a result of injections of tubercle bacilli from the turtle. The reports up to the present warrant no especial consideration and his work is only mentioned because it has attracted so much attention in this country. In Germany where the first work was done and reported the glowing reports are looked upon with distrust.

Autogenous vaccines and tuberculins have been recommended and used by some. Whenever injections of tubercle bacilli are made it should be remembered that they are little absorbed and that a nodule may remain at the site of injection. Dixon¹ has claimed to produce resistance to lethal doses of virulent tubercle bacilli by injection of fat-free tubercle bacilli and branching non-acid forms.

Vaccines and sera to overcome organisms causing mixed infection in tuberculosis are of value in some cases, though as has already been stated repeatedly the organisms causing the mixed infection should be definitely identified before any mixed vaccines or sera are

¹Dixon: J. Am. Med. Ass., 1913, LX, 993.

given. Furthermore infection with the other organisms frequently disappears after the tuberculous process is healed.

Serum Therapy and Passive Immunization.—Serum treatment and immunization have long been considered. Some of the means used in immunization have been regarded as methods of serum treatment but really have not been such. Koch's old tuberculin was called Koch's lymph though as has already been shown it is not a serum or any other body fluid. It must be evident that little can be hoped from specific serum treatment for the blood of man and the animals having tuberculosis shows little increase in the known antibodies. Recovery from the disease is not associated with antibody formation but rather with processes leading to incapsulation of the tubercle bacilli. Serum treatment for tuberculosis has not been confined to the use of antisera for the serum of certain animals has been regarded as possessing an especial curative value. Of the specific antituberculosis sera those of Maragliano and Marmorek and Neporoschny are best known. Maragliano immunized horses for four to six months to toxins prepared by the filtration of cultures only a few days old and to those obtained by aqueous extraction of killed virulent cultures. He assumes that this serum contains antitoxic, bactericidal and agglutinating properties and in addition in man is able to stimulate the body to produce new specific protective substances. One cubic centimeter of this serum is injected every other day for one and a half months. Favorable reports have come from Italy but in Germany and France proof of its value could not be established. Marmorek, Behring and others have advocated the use of milk of cattle immunized to tuberculosis.

Serum from the goat has long been supposed to possess a curative value especially when the serum can be locally applied. It apparently has been of little value as a therapeutic measure.

Prophylaxis.—The general problem of prophylaxis against tuberculosis has been much discussed and will not be entered into here. Immunization against the disease is being advocated especially in children predisposed and more especially exposed because of tuberculosis of the parents or immediate family. Various methods for immunization have been advanced and include injections of tuberculin, killed virulent and living avirulent cultures of the tuber-

cle bacillus. More recently attempts have been made at immunization with extractives of the bacilli and culture medium on which they have been grown. Immunization of the predisposed and especially exposed will undoubtedly gain more favor and while the methods are not entirely established it is well for the physician to watch closely patients who have been exposed so that specific measures for treatment may be instituted at the first indication of the disease. Immunization of cattle has certainly been of value and at least indicates and points out lines for attempts at the immunization of man. The disease is undoubtedly conveyed from some of the animals to man though of course the spread is far more general from the human than animal sources. It is advisable that meat and milk from animals not known to be free from tuberculosis be exposed to such heat as will surely kill tubercle bacilli.

INFECTIONS WITH BACT. MALLEI (Glanders Bacillus).

Glanders is a disease occurring principally in horses and asses. Because of the susceptibility of man, the high death rate in man and his intimate association with the infected animals the disease is of considerable importance to us. The disease occurs in two principal forms. One is known as glanders and has its principal lesions especially in the mucous membranes of the nose; the other is known as farcy and manifests its lesions principally in the skin. The organism of glanders was discovered by Loeffler and Schütz.

Differential Specific Diagnosis.—The diagnosis of glanders must be made in animals and in man. In both it occurs as acute and chronic glanders and as acute and chronic farcy. The chronic form occurs more frequently in horses than does the acute form.

CLINICAL.—The chronic forms in the horse are often not recognized but any horse having a purulent discharge from the nose or nodules in the skin, especially near the extremities, should be suspected and subjected to bacteriological examination. The acute form in horses is more easily recognized and usually terminates in death. In these cases ulcers develop in the mucous membrane of the nose and all over the body are painful swellings that have a tendency to break down.

In man the disease appears after an incubation period of four to eight days and always within three to four weeks. The patient seldom has a chill but has irregular fever and pains in the limbs and head. In the glanders type on the mucous membranes of the nose and throat appear crater-like pustules filled with sero-purulent material and having a tendency to break down in the center and to form an elevated border about the ulcer. Usually also the outer skin is involved. There is first an exanthema of small red spots and later in various parts of the body nodules are formed in the deeper layers of the skin and muscles. These become fluctuating and on incision show a gelatinous, dark, purulent material. Similar areas may develop in the lungs. Death is invariable and in acute cases follows in eight to ten days and in two to three months in chronic cases. One of the cases seen by the author was first diagnosed as syphilis, later as smallpox and only finally as glanders. The best diagnosis is based on bacteriological examination.

BACTERIOLOGICAL DIAGNOSIS.—For the bacteriological diagnosis of glanders subcutaneous or intraperitoneal injections of the pustular material or blood should be made into *male* guinea-pigs. Following such injections the so-called Straus reaction appears. In this the testicles become swollen and on incision show areas with purulent exudate. Other organisms also produce enlargement and necrotic areas in the testicle, but the test is of value as it is accurate enough to suspect glanders and when the reaction does not occur glanders can be excluded. Inoculation of male guinea-pigs cannot be overemphasized. In one case that came under my care the diagnosis was missed for some time because female guinea-pigs only were inoculated. From the pus of the testicle cultures should be made. The organism is non-motile, does not hold Gram's stain and when stained with methylene-blue frequently shows polar bodies. The growth is usually heavy and tenacious. The organisms should be tested for agglutination with specific antisera. For further cultural characteristics the text books are referred to.

SERUM DIAGNOSIS.—Agglutinins and bodies possessing the ability to fix complement are developed during the disease. The macroscopic method for agglutination is best and should be positive in dilutions of 1 to 500. The agglutination test is also of value in

the differentiation of glanders bacilli from organisms resembling it. Arms has employed the fixation of complement reaction in the diagnosis of glanders and finds it of assistance in most cases.

HYPERSENSIBILITY TEST FOR DIAGNOSTIC PURPOSES.—Under the name of mallein various preparations used for diagnostic purposes exist. Animals infected with glanders bacilli are much more susceptible to these malleins than are normal animals. The reaction is manifested in several hours and lasts for 30 to 40 hours. The original mallein of Roux and Nocard was made after the manner of old tuberculin, 2 to 8 weeks' cultures on glycerin bouillon being sterilized by heating and the bacteria removed by filtration and the filtrate reduced to one-tenth its volume. Other preparations are: Helman's extract of the bacilli with glycerin and water, Kalning's aqueous extract, alcoholic precipitates from the filtered culture as was done by deSchweinitz, and so on. Morvin and dried mallein are dried precipitates. The dose for the different preparations is indicated on the package and usually varies between 0.2 and 0.4 c. c. The test is practically only applied in animals. There is only a transitory local reaction and entire reliance must be placed on the temperature reaction.

The value of the test is doubted by some but when it is used in conjunction with the serum tests and if possible with the bacteriological test, it is of great importance.

Immunity and Specific Therapy.—Man possesses no immunity to the disease. Horses, asses, cats and guinea-pigs are especially susceptible. Sheep and goats have some resistance and cows are immune. During the disease antibodies are formed but they are of no value in passive immunization. Active immunization has proven of no value. Mallein has not the beneficial effect that tuberculin has in tuberculosis. In horses recovery apparently occurs at times but in man practically never. There is no acknowledged specific cure¹.

¹Several years ago I saw in consultation a child with advanced glanders. A sheep that had received repeated injections of mallein being available, blood was drawn from it and the serum used for injection into the child. At intervals of five to six days five doses of 5 c. c. each were injected. Under these injections the child's condition improved markedly and it was believed the child would recover. Unfortunately the physician in charge of the case allowed the family to persuade him to discontinue the serum injections. Two weeks after this the condition again became aggravated and serum was again injected. The child however had developed such marked hypersensitivity to serum that serum injections could not be continued. Injections of killed cultures of glanders bacilli were tried but to no avail and the child died six weeks after the serum injections were discontinued. No further opportunity to try such serum therapy has since presented itself to me.

Prophylaxis.—For the prevention of the disease in man early diagnosis of the disease especially in horses is essential. Horses with discharge from the nose or having nodules in the skin should be tested with the mallein and serum tests. All diseased animals should be killed and cremated at once. The number of horses with glanders in cities periodically attracts considerable attention but in the interim little attention is given to this highly communicable and fatal disease. When the disease occurs in man strict quarantine should be instituted. Workers in laboratories should be careful in handling tissues and materials from glanders-infected animals or humans and should be careful in the handling of glanders cultures. Numerous infections have occurred in veterinarians and laboratory workers and in most cases have soon terminated fatally.

INFECTIONS CAUSED BY BACTERIA BELONGING TO THE TYPHOID AND COLON BACILLUS GROUP.

A considerable number of cases of the more frequent intestinal diseases are caused by bacteria closely resembling each other. Clinically these diseases are divided into typhoid fever, bacillary dysentery and intestinal intoxication. It is found however that even the clinical entities are caused by different bacterial varieties. Thus clinical typhoid fever may be caused by *Bac. typhosus*, *Bac. paratyphosus A* or *Bac. paratyphosus B*. Bacillary dysentery may be due to one or more of the several varieties of *Bact. dysenteriae*. Intestinal intoxication, in addition to other species of bacteria, is associated at times with *Bac. coli* or *Bac. enteritides* of Gærtner belonging to the particular group of bacteria considered here. The various diseases clinically diagnosed and having specific etiological factors in this group, in many cases are modified and influenced by other organisms as *Strep. pyogenes*, *Mic. pyogenes*, the gas bacillus and so on.

In the chapters on Immunity and Specific Diagnosis it was pointed out that the antibodies first formed and produced in largest amounts are specific while the later ones are common to closely allied species. Realizing that this group contains closely related organisms it must be remembered that in the serum diagnosis of the etiological factor

in these diseases there may be antibodies for any of the organisms of this group late in disease or years after recovery and even in adults that have had repeated disturbances due to *Bac. coli*. This may lead to error in the diagnosis unless the case is properly studied and controlled.

TYPHOID FEVER.

Typhoid fever usually occurs in the form of abdominal typhoid. The organisms causing clinical typhoid fever are also able to and do produce infection and disease in the various parts of the body, the principal ones being peritonitis, appendicitis, meningitis, pneumonia, bone abscesses, otitis media, and so on. While these conditions usually occur during or after abdominal typhoid fever, they may occur without the presence or at least the diagnosis of abdominal typhoid.

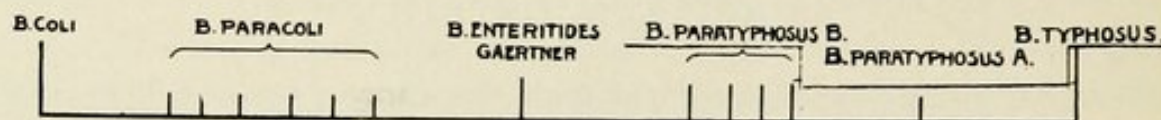


Fig. 18.—Schematic drawing to show relation of bacteria of the typhoid and colon group.

The etiological factor in the disease is the typhoid bacillus. In more recent years due largely to the efforts of Schottmüller it has been found that other species also produce conditions similar to those caused by the typhoid bacillus. These organisms are referred to as paratyphoid bacilli and are closely related to *Bac. typhosus*. While there are many more variations, the general groups of these organisms are related as shown diagrammatically in Fig. 18.

The various causes of what is clinically known as typhoid fever are found in the circulating blood early in the disease, after eight to ten days produce agglutinins sufficient to give positive agglutination (Grüber-Widal) reactions, are present in the stool practically throughout the disease, may be present in the urine during the disease and may be present in the stool and urine long after recovery, thus making the patient a so-called "typhoid-carrier." While these organisms usually produce what is known as abdominal typhoid they may produce pathological conditions in the peritoneum, lungs,

nervous system and so on, either during or in conjunction with or independent of abdominal typhoid.

Differential Specific Diagnosis.—CLINICAL.—The diagnosis of typhoid fever and infections with the typhoid bacillus group of bacteria must be based on clinical as well as on specific factors. The usual clinical symptoms need not be considered here. There are no certain distinguishing points by which to separate abdominal typhoid and paratyphoid clinically. Paratyphoid fever is more likely to have a shorter incubation period, a sudden rise in temperature, to give the symptoms of acute gastro-enteritis, a better prognosis and more patients when discharged are still "carriers."

BACTERIOLOGICAL DIAGNOSIS.—The best diagnosis of the infection is based on the isolation and identification of the specific organisms. During the disease the micro-organisms belonging to the typhoid group may be obtained from various sources. In which of the materials they are to be searched for depends to some extent on the duration of disease when the study is made. Because the organisms at times are only present in small numbers or are accompanied by large numbers of other bacteria, a medium especially favoring the growth of this group of organisms is of great advantage. The medium most frequently used is sterile ox bile which after inoculation is held at 37° C. for 12 to 24 hours when the regular plate cultures are made.

(a) *Blood Cultures.*—These furnish the earliest positive diagnosis of infection with typhoid bacilli. Cultures are obtainable long before the agglutination reaction occurs. During the first week of the disease Brion and Kayser obtained 100 per cent. of positive cultures. During the second week the bacilli are found in about 60 per cent. and during the third to fifth week in about 40 per cent. of the cases of the disease. In a few cases the bacilli have been found in the blood during convalescence. Blood for the examination must be obtained under aseptic conditions, the methods for the same having been described on page 37. Miller and Graef have also obtained cultures from the blood clot.

(b) *Roseolar Spots.*—Fresh roseolæ must be selected as the bacilli are dead in the older ones. The skin should be sterilized in the ordinary manner and with a scalpel or curette the epithelium and serum transferred to bouillon or bile, incubated and examined.

Various investigators have obtained positive results in 80 to 90 per cent. of the cases examined.

(c) *Feces Examination*.—Jürgens, v. Drigalski and Ford have found that typhoid bacilli are found in largest numbers in the upper duodenum. Lower down in the tract they become less numerous, the cæcum and rectum frequently containing none. They occur in the stomach, mouth and in the saliva. Many media have been advocated for the isolation of typhoid bacilli from stools but of all it may be said that there is no medium available at present that retards the growth of all other bacteria or one that favors only the multiplication of typhoid bacilli. Usually however ox bile is regarded as the best enriching medium. From this medium plate cultures must be made in 12 to 24 hours. The plate medium used varies in the different laboratories. Of these only a few need be given here:

HISS' PLATE MEDIUM.—

Agar 10 grams.
Gelatin 25 grams.
Beef Extract 5 grams.
Sodium Chloride 5 grams.
Dextrose 10 grams.
Water 1,000 c. c.

This is titrated with phenolphthalein to 2 per cent. acidity. Colonies of motile bacilli are irregular in outline, being fringed. Examination for this is made under a low-power microscope.

MODIFIED ENDO MEDIUM (KENDALL).—The modification of Endo's medium as recommended by Kendall and Walker¹ is of great help. For the details of preparing the medium the article should be consulted. The medium consists of water 1,000 c. c., agar 30 grams, and peptone 10 grams. These are heated until dissolved, then neutralized with sodium carbonate using litmus as the indicator. After this 10 c. c. of a 10 per cent. solution of sodium carbonate are added and the medium is sterilized. When plates are to be made 2 c. c. of a 10 per cent. basic fuchsin solution in 90 per cent. alcohol are added to 10 c. c. of a 10 per cent. solution of sodium sulphite in water which has been sterilized in an Arnold

¹Kendall and Walker: J. Med. Research, 1910, XXIII, 481.

sterilizer. Of this mixture 0.5 c. c. and 1 gram of chemically pure lactose are added to each 100 c. c. of the molten agar solution. The plates are then inoculated and sufficient agar poured into them. Organisms not fermenting lactose, of which *B. typhosus* is one, are colorless while those fermenting lactose become red. Other media used are those advocated by Drigalski and Conradi¹ and by Grünbaum and Hume.²

Of all media of value in the separation of organisms producing colonies similar to those produced by the typhoid bacillus, the tube medium of Hiss is recommended. It consists of agar 5 grams, gelatin 80 grams, beef extract 5 grams, sodium chloride 5 grams, dextrose 10 grams, water 1,000 c. c. It is titrated to 1.5 per cent. acidity, using phenolphthalein as the indicator. In this medium gas and motility are detected by bubbles of gas and clouding respectively.

Typhoid bacilli may also be found in the various organs, in the bile, urine and at times in the cerebrospinal fluid.

Identification of typhoid bacilli is made according to the usual bacteriological methods. The differentiation of *Bac. typhosus*, *Bac. paratyphosus A*, and *Bac. paratyphosus B* is based on the agglutination test, Pfeiffer's reaction and fermentation of different carbohydrates.

Both *Bac. paratyphosus A* and *B* produce acid and gas in dextrose in this way resembling *Bac. coli*, while they resemble *B. typhosus* in not causing coagulation in litmus milk. Culturally *Bac. paratyphosus A* is differentiated from *Bac. paratyphosus B* by the failure to produce real alkalinity and solution of the casein. The best means of differentiation of the organisms of this group is by the agglutination test and by the Pfeiffer reaction.

Serum Diagnosis.—The agglutination reaction has been utilized most extensively for the diagnosis of typhoid fever in man. The test is however not limited to the differential diagnosis of typhoid fever from other diseases but also for the differentiation of closely related organisms of the typhoid-colon group. This group contains a number of organisms widely distributed in nature, all able

¹Drigalski and Conradi: Ztschr. f. Hyg. u. Infektionskrankh., 1902, Heft 11, 283.

²Grünbaum and Hume: Brit. M. J., 1902, Pt. I, 1473.

to cause digestive disturbances under conditions frequently present in the digestive tract. These organisms are capable of producing specific and group antibodies. The specific antibodies produced during disease or in active immunization appear first but gradually the group antibodies appear and all persist for some time afterward. In utilizing the agglutination phenomenon as a diagnostic measure we must therefore take into consideration that small amounts of specific and group agglutinins occur without the disease, that a former attack of typhoid fever may leave enough agglutinins to give a reaction for some years, that the reaction may not be positive until after the first week of the disease and that late in the disease the reaction may be positive with any of the micro-organisms in this group.

Agglutination Test for Diagnostic Purposes.—For this the microscopic test is used quite universally. The technique varies in different laboratories. Good results are obtained with both dried blood and with serum. Because of common agglutinins and the clinical resemblance of typhoid fever and paratyphoid fever, agglutination tests should be made with suspensions of *B. typhosus* and *B. paratyphosus* A. and B. The importance of this cannot be over-emphasized. Merely using suspensions of *B. typhosus* in the tests has discredited the value of agglutination in diagnosis of typhoid fever. The dilutions of serum used in the test vary from 1:10 to 1:100. It is a quite generally adopted rule that for a positive diagnosis there must be complete clumping of bacteria in one-half to one hour using serum dilutions of 1:40 or 1:50. In all cases there must be full sets of control preparations.

In interpretation of the result of the test various things must be considered.

1. Specific agglutinins appear first and in largest amounts, so that infection is due to the particular variety agglutinated in the highest dilution of serum. At times this can be determined by subsequent tests in which the reaction occurs in higher dilutions than in the earlier tests.

2. The reaction is often not positive before the second week of the disease so that if the clinical symptoms are those of typhoid fever further agglutination tests must be made. Agglutination is usually at its height in the third and fourth week of the disease.

The appearance of and amount of agglutinins are apparently of no prognostic value.

3. At times there is a partial reaction, that is there is cessation of motility or there is some clumping. Again the reaction may be positive in 1:10 dilutions but not in the higher dilutions. In these cases it is best to regard the reaction as "suggestive" and to repeat tests every one to two days.

4. Agglutination may occur because of a former attack of the disease or because of active artificial immunization. Usually the reaction is negative for four to five months after the attack and in children even earlier. Cases have been reported in which the reaction has persisted for years. Agglutination due to active immunization (vaccination) persists for one to two years.

5. Agglutination may be positive in patients who have not had typhoid fever. Serum of jaundiced persons frequently agglutinates typhoid bacilli. This agglutination seems to be quite specific for typhoid bacilli. It is possible that in jaundiced patients the jaundice may be due to infection with organisms producing specific and common agglutinins.

6. Not all persons having infection with typhoid bacilli have agglutinins in the blood serum. This failure to produce agglutinins is rare, Rostoski observed it three times in 220 cases in the medical clinic in Würzburg.

7. A strongly positive agglutination reaction without the symptoms of abdominal typhoid should lead us to suspect an unusual location of the infection as in the lungs and so on.

8. While the agglutination test is in no way to be regarded as infallible or conclusive it must be ranked first in importance as a symptom of typhoid fever.

OPHTHALMIC TEST.—Chantemesse¹ using his ophthalmic test obtained a positive reaction in 70 cases of typhoid fever and a negative reaction in 49 out of 50 persons not having typhoid. Floyd and Barker found a positive reaction in 96 per cent. of patients with typhoid fever and in 16 per cent. of those not having the disease. Austrian realizing that the results so far obtained are not accurate enough made an extract from eighty strains of *B. typhosus*. He grew these in bouillon from which he sedimented them by centri-

¹Chantemesse: Deutsche med. Wchnschr., 1907, XXXIII, 1246, 1572.

fugalization, washed them and then killed them by heating to 60° C. for two hours. The killed bacteria he dried and ground up with salt, then macerated them in water after which the solution was precipitated with absolute alcohol. The residue he dried, pulverized and dissolved 10 milligrams in 1 c. c. of water. This he dropped into the lower conjunctival sac. A positive reaction appeared in 6 to 10 hours. Austrian¹ found the reaction positive more frequently than the agglutination tests or blood cultures and furthermore got positive results as early as the second and third days of the disease. This test will need further study. Differentiation between *Bac. typhosus* and *Bac. paratyphosus* in these tests should certainly be made.

CUTANEOUS TESTS.—Cutaneous tests for the diagnosis of typhoid fever have been advocated. The material injected has consisted either of killed bacilli or some of their extracts. The method of making the tests is similar to that for tuberculosis, syphilis and so on. Szontagh² only observed a positive reaction in four of seventy-two children tested with typhoid toxin.

RESUMÉ ON DIAGNOSIS.—Any of the laboratory tests for diagnosis of these diseases should only have the value of a cardinal symptom. The presence or absence of the bacilli, agglutination, and so on do not positively speak for or against the existence or absence of the disease. In order to make the different laboratory tests of the greatest value it must be remembered that there are a number of bacteria that produce clinical typhoid fever and in all tests at least *B. typhosus*, *B. paratyphosus A.* and *B. paratyphosus B.* should be tested for. Even finding the bacilli does not necessarily mean that the patient has the disease for an individual may be a carrier and because of this be a menace to the community. It must also be remembered that these bacilli cause infection in the various parts of the body and that these infections are likely to occur during or after the regular course of abdominal typhoid fever.

Immunity and Specific Therapy.—After an attack of typhoid fever there is generally supposed to be a period during which there is immunity. In the blood of patients recovering from typhoid

¹Austrian: Johns Hopkins Hosp. Bull., 1912, XXII, 1.

²Szontagh: Arch. für Kinderheilk., 1912, LVIII.

fever agglutinins are present for a considerable period of time. Whether there is actual immunity or only a lack of vulnerable tissue in the intestines is an open question. To a certain degree the existence of immunity must be doubted when we consider the frequency of post-typhoid infections and the number of typhoid carriers. Experimental immunization of animals results in the production of antibodies and in some cases an apparent protection. The principal antibodies produced by active immunization are agglutinins, bacteriolysins and opsonins. The length of time for which antibodies persist varies but the protective bodies following active immunization according to the experiments of Wollstein¹ are seldom present for longer than one year. The body cells after active immunization are however sensitized so that they respond more quickly to subsequent infections or injections.

Vaccine Therapy and Active Immunization.—The first work on experimental protective active immunization against typhoid was published in 1896 by Pfeiffer and Kolle, and in 1897 by Wright. For a number of years active immunization against the disease has been practiced by the English and Germans. The method of preparing the vaccine has differed to some extent in these two countries. Wright used 24 to 48-hour bouillon cultures standardized to contain a certain number of bacilli to the cubic centimeter, and Pfeiffer and Kolle used platinum loop-fulls of 18 hours' growth on agar. According to both methods the bacilli are killed by heat. Pfeiffer and Kolle repeated injections at intervals of about eight days until four doses were given, while Wright in the beginning used only one injection and later advocated giving two smaller doses. In this country various methods have been advocated and while they differ in some details they conform quite closely to those advocated in Europe and by the medical officers in the United States Army. Extensive antityphoid vaccination began in the United States in 1908.

The preparation of the vaccine is relatively simple. Typhoid bacilli are grown on agar for 24 hours at 37° C. when they are washed off with sterile salt solution, the solution standardized, the bacteria killed by heating for 30 to 60 minutes at a temperature of 55° to 60° C., and then is added 0.25 per cent. tricresol or lysol.

¹Wollstein: J. Exper. M., 1912, XVI, 315.

Those immunizing in the United States army have used a single strain of typhoid bacillus, others have used various strains and Kabeshima¹ and others have advocated the use of vaccine containing typhoid and paratyphoid bacilli. Metchnikoff and Besredka² believing that many bacteria are necessary to produce immunity have advocated the injections of living typhoid bacilli basing their conclusions on experiments in chimpanzees. Injections of living cultures of typhoid bacilli however produce such marked reaction that these men advocate injections of bacilli exposed to the action of heated ox serum. With sensitized bacilli they claim to get equally good protective action in the chimpanzee. Castellani has injected living bacilli attenuated by exposure to 50° C. To the use of living organisms there has been much objection because of the fear of producing the disease and typhoid carriers. Killed cultures have been used most generally, are efficient as a preventive measure and are to be advocated at this time.

The dosage of and interval between injections has varied considerably. The great variation, however, is partly dependent upon the object of immunization, that is whether the injections are made for prophylactic or curative purposes. For protective immunization much larger doses are given than for a curative purpose. The approximate doses for protective immunization are one hundred million at the first injection, then two hundred million, four hundred million and six hundred million at intervals of five days. In the United States army three injections are given at seven to ten day intervals, the first dose being five hundred million and the second and third one thousand million bacilli. For curative purposes the doses vary but generally are not so large. Sadler³ uses very small doses, one to two million and decreases these on the second injection four to five days later. Walters and Eaton⁴ have used larger doses even to one hundred and five hundred million bacilli. The doses most frequently used are from ten to fifty million bacilli injected at five to seven day intervals.

As a result of typhoid vaccine injections certain local and general reactions are obtained at times. The local reactions are tenderness, pain and hyperæmia. The regional glands are enlarged and tender.

¹Kabeshima: Naval Med. Assoc. of Japan Bull., 1912, May 1.

²Metchnikoff and Besredka: Ann. de l'Inst. Pasteur, 1911, XXV, 461.

³Sadler: Quart. Jour. of Med., 1912, V, 193.

⁴Walters and Eaton: Boston Med. and Surg. Jour., 1909, CIX, 503.

These reactions come on in six to eight hours and last two or three days. The general reaction consists of malaise, headache, fever to 102-103° F., vomiting and at times diarrhoea. They last from 24 to 48 hours. During menstruation the symptoms are more likely to be severe. Injections for prophylactic purposes should not be made during menstruation or when there is debility, arthritis, urethritis, cholecystitis, and so on. The first and last injections are less likely to produce reactions than the second and third and in patients having had typhoid fever all of the reactions are more likely to be severe. When injections are given for curative purposes the doses should be so small as to avoid or give only the slightest reactions.

The changes in the blood as a result of typhoid vaccination have been much considered. Albert and Mendenhall find that typhoid vaccination and typhoid fever have in common the increase in large mononuclear cells and believe that they have some relation to typhoid immunity. On injection of proper doses of typhoid vaccine, agglutinins, opsonins and bacteriolysins are produced. Broughton-Alcock finds that when vaccination is made with living cultures the serum does not contain complement-fixation bodies and seldom agglutinins. He however believes that this does not indicate the absence of immunity. Immunity as a result of typhoid vaccination is believed to last from one to three years. If this is true then Wollstein's¹ observations that the immune bodies disappear within 10 to 15 months indicate that clinical immunity cannot be determined by laboratory methods. She suggests that there may be a latent power of the body cells to react more quickly to a stimulus that has once sensitized them.

The results of active immunization have been valuable especially as a prophylactic measure. This has been particularly noticeable in armies and among nurses and physicians in hospitals. The curative value has been less definitely decided. Chantemesse in 1907 at the International Congress of Hygiene and Demography reported the results of his treatment of typhoid fever with killed cultures. The death rate dropped from 17 per cent. to 4 per cent. and 75 per cent. were improved by the treatment. Walters and Eaton reduced the case mortality from 11.1 per cent. to 3.2 per cent. The

¹Wollstein: J. Exper. M., 1912, XVI, 315.

curative value of active immunization however needs further investigation over longer periods of time. It must be acknowledged that in some clinics this treatment has been of definite value.

The best results with vaccine have been obtained in cystitis, cholecystitis and the local abscesses following attacks of typhoid fever. The doses injected in these cases range usually from five million to fifty million bacilli, injections being repeated at intervals from eight to ten days. Autogenous vaccines usually give the best results.

Serum Therapy and Passive Immunization.—Before the discovery of the organisms causing typhoid fever it was known that an attack of typhoid fever usually gives some protection against a second attack of the disease. After the discovery of the typhoid bacillus it was found that animals repeatedly injected with non-fatal doses of this organism will ultimately be protected against otherwise fatal doses. It was also found that by injecting blood serum from immunized animals, a passive immunity against typhoid bacilli can be conferred to other animals. Based on these results various anti-typhoid sera have been made for the specific treatment of the disease of typhoid fever in man.

Antityphoid sera are usually obtained from horses which have been immunized either by repeated increasing injections of dead and living cultures of typhoid bacilli or typhoid bacillus toxins. Most of the sera prepared have not been antitoxic but antibacterial. Chantemesse has prepared a so-called antitoxic serum by injecting horses with increasing doses of pure cultures of typhoid bacilli grown for some time on a macerated splenic pulp and defibrinated human blood medium. Tavel by repeated injections of two weeks' old bouillon cultures, sterilized by the addition of 0.5 per cent. carbolic acid, has made a serum which is supposed to possess antitoxic substances. Most of the sera are however made by injecting into horses and other animals increasing doses of dead and living typhoid bacilli of various strains. For these sera little or no antitoxic value is claimed, the immunizing properties being dependent on the lytic and opsonic powers of the serum.

In the treatment of typhoid fever with these sera, 10 c. c. and frequently larger quantities are injected daily until improvement occurs.

The results following the use of antityphoid serum have been disappointing. In most cases no effects on the course of the disease have been observed. Chantemesse in 1902 reported that by the use of his serum the death rate in children due to typhoid fever was 3 per cent., whereas the death rate in all the children of Paris who received no such serum injections was 19 per cent. Occasionally observers have obtained and reported cases in which improvement is rapid after the injection of antityphoid serum. In some cases a drop in the fever and improvement in the pulse and general conditions have been reported as resulting from the use of this serum. Various reasons have been assigned for the failure of the curative action of the serum, the most important of which are the lack of antitoxic substances which can combine with the toxin liberated after solution and disintegration of the bacilli due to the specific lytic substances in the serum and the failure of the body to supply a sufficient amount of complement so as to get the destruction of the invading typhoid bacilli.

Jez has prepared an extract from the spleen, bone marrow and lymph glands of animals immunized to typhoid bacilli. This extract is usually administered by the mouth and is supposed to possess antitoxic properties. The value of this extract is doubtful.

Antityphoid sera are still in the experimental stage. With the sera now produced no marked beneficial results can be hoped for and they are only used in certain severe cases of the disease.

Prophylaxis.—Typhoid and paratyphoid infections in most cases have their portal of entry in the digestive tract and in most cases the organisms leave the body through the feces and urine. As far as contraction of infection is concerned, preventive measures deal almost entirely with prevention of ingestion of contaminated foods. These means while efficient have failed in a large measure because we cannot always know when food is contaminated. Prophylactic immunization of men in armies, physicians and nurses in hospitals and other people especially exposed has already been mentioned in the consideration of active immunization against typhoid fever. The protective value cannot be doubted and the practice of actively immunizing all people exposed to typhoid infection can be advocated conscientiously. The duration of immunity generally is for two or three years still it is probably

best to immunize again after a year if patients are exposed to infection as in armies, hospitals and so on. For the present immunization with killed cultures is recommended although consideration must be given to immunization with sensitized living cultures as is advocated by Metchnikoff and Besredka and Broughton-Alcock.

Protective immunization partially destroys our means of serum diagnosis of infections with typhoid and paratyphoid bacilli because the antibodies persist for some time. The value of these tests is however not entirely destroyed. Positive agglutination of typhoid or paratyphoid bacilli by the serum of a patient having been immunized does not indicate infection unless the agglutinins increase from day to day. For this reason tests to determine the greatest dilutions of the patient's serum that will cause agglutination should be made on several days.

INFECTIONS WITH BACTERIUM DYSENTERIÆ.

Dysentery for a long time included all of the diseases of the intestines in which there is frequency of stool. Later the term was limited to diseases in which there is necrosis and ulcer formation in the intestine and finally two forms of dysentery were recognized, one an epidemic dysentery and the other an endemic dysentery. Various different organisms have been associated with these diseases. In 1873 Loesch was able to find amoebæ in the ulcers of certain cases of dysentery and since that time the type of amoebic dysentery has been definitely established. Amoebæ however could not be held responsible for all of the dysenteries so that further investigations were necessary. Of the early work that of Booker¹ is the most exhaustive in the United States. In 1896 Shiga determined that what is now known as *Bact. dysenteriae* is the causal organism in Japanese dysentery. This was followed by the work of Flexner in 1900 on tropical dysentery in Manila and the researches by Kruse in 1901 on epidemic dysentery in Germany. The investigations of Flexner and Kruse confirmed the results of Shiga. In 1902 Vedder and Duval isolated the organisms in this country and in the summer of 1902 Duval and Bassett definitely established *Bact. dysenteriae* as the etiological factor in some of the summer diarrhœas of infants in the United States. From that time

¹Booker: Arch. of Pediatrics, 1890, February; also Johns Hopkins Hospital Reports, 1896, VI, 159.

on *Bact. dysenteriae* has been isolated from the dejecta in dysentery in various parts of the world.

While all of the organisms at first isolated were regarded as conforming in all characteristics with the organisms isolated by Shiga it was noted by McConkey and Hill¹ that organisms isolated by Shiga do not ferment mannite while those isolated by Flexner do. These men however were not making a special study of dysentery bacilli so these results were not referred to in their text. In 1902 Martini and Lentz and in 1903 Hiss and Russell brought the differences out clearly. There now are established two definite groups of dysentery bacilli: those not fermenting mannite and those fermenting mannite. It was found that in addition to this difference there is a further important difference; the Shiga type not fermenting mannite produces a soluble toxin while the Flexner-Harris type, which does ferment mannite, does not produce a soluble toxin.

The mannite fermenting group has been further divided on the basis of fermentation of the carbohydrates. Based on a study of 200 isolations, the fermentation properties of which were studied in Hiss' serum-water medium, Knox and Schorer² divide dysentery bacilli as follows:

5 DAY FERMENTATION.

	I SHIGA	II HISS "Y"	III STRONG	IV FLEXNER- HARRIS	"B" DUVAL & SCHORER
Dextrose.....	Coag.	Coag.	Coag.	Coag.	Coag.
Levulose.....	Coag.	Coag.	Coag.	Coag.	Coag.
Galactose.....	Coag.	Coag.	Coag.	Coag.	Coag.
Mannite.....	Neg.	Coag.	Coag.	Coag.	Coag.
Lactose.....	Neg.	Neg.	Neg.	Neg.	Neg.
Saccharose.....	Neg.	Neg.	Acid	Coag.	Neg.
Maltose.....	Neg.	Neg.	Neg.	Coag.	Coag.
Dextrine.....	Neg.	Neg.	Neg.	Coag.	Coag.
Litmus Milk.....	Neg.	Neg.	Neg.	Neg.	Neg.

25 DAY FERMENTATION.

	I SHIGA	II HISS "Y"	III STRONG	IV FLEXNER- HARRIS	"B" DUVAL & SCHORER
Dextrose.....	Coag.	Coag.	Coag.	Coag.	Coag.
Levulose.....	Coag.	Coag.	Coag.	Coag.	Coag.
Galactose.....	Coag.	Coag.	Coag.	Coag.	Coag.
Mannite.....	Neg.	Coag.	Coag.	Coag.	Coag.
Lactose.....	Neg.	Neg.	Neg.	Neg.	Acid
Saccharose.....	Neg.	Coag.	Coag.	Coag.	Acid
Maltose.....	Acid	Coag.	Coag.	Coag.	Coag.
Dextrine.....	Neg.	Neg.	Neg.	Coag.	Coag.
Litmus Milk.....	Neg.	Neg.	Coag.	Neg.	Acid

¹McConkey-Hill: Thompson Yates Lab. Reports, 1901, IV, Part 1, 160 (table).

²Knox-Schorer: Jour. Exper. Med., 1906, VIII, 377; also Johns Hopkins Hospital Reports, 1904, XV, 1.

Group I.—Ferments the monosaccharids only during the first five days, but later also ferments maltose; however, not producing a coagulum in the serum-water. This group is represented by the Shiga culture sent to this country.

Group II.—Ferments monosaccharids and mannite early while after twenty days saccharose and maltose serum-water are coagulated. This group is represented by the Hiss and Russell "Y" organism.

Group III.—Ferments early the monosaccharids, mannite, and saccharose, while maltose serum-water is coagulated after twenty-five days. This group is represented by the Strong culture.

Group IV.—Ferments the monosaccharids, mannite, maltose, dextrine and saccharose readily. This group is represented by the Flexner-Harris culture.

All of the groups are closely related and produce specific and common agglutinins as will be shown later.

Differential Specific Diagnosis.—CLINICAL.—Dysentery in the United States and practically all temperate climates occurs in epidemic form only in children and institutions especially those for the insane, while in tropical climates it exists among all classes and at all ages. In children infection with *Bact. dysenteriae* occurs under a variety of conditions. It occurs as an acute infection in previously well children, as a subacute infection following acute symptoms, with or following other acute diseases or as a terminal infection in marasmus and malnutrition. It also occurs at times in cases having only mild intestinal disorders. Fever may be marked or absent. It occurs in breast fed and artificially fed children. Knox and Schorer in a study of seventy-four cases of children's dysenteries found dysentery bacilli in 73.1 per cent. of the cases. The 74 cases were divided clinically as follows: inflammatory colitis, 29 cases; toxic, dyspeptic and fermental diarrhoea, 36 cases; intestinal indigestion, 7 cases; chronic gastroenteritis, 1 case; tuberculous ileo-colitis, 1 case. From all of the investigations on children's diarrhoea it is safe to assume that there is infection with *Bact. dysenteriae* if the stools contain macroscopical or microscopical pus. Infection with the Shiga type of dysentery bacillus is most severe although infection with any of the types may be as severe especially when streptococci also are present. In the investigations of Knox and Schorer the Hiss Y type occurred most frequently. Frequently the disease follows error in diet and is ushered in by vomiting. The disease is primarily one occurring in the hot months of the year.

BACTERIOLOGICAL DIAGNOSIS.—The isolation of dysentery bacilli alone does not establish the existence of bacillary dysentery for the organisms have been isolated from the stools and intestinal mucosa scrapings of normal children. The history of the illness is indispensable in determining the significance of the presence of *Bact. dysenteriae*. Likewise failure to find the organism does not exclude bacillary dysentery. The technique is such that diligent search may be necessary. The 412 cases of children's diarrhoeas studied by the Rockefeller Institute¹ in 1903 show that the percentage of positive findings as obtained by the eight groups of investigators varied from 25 per cent. to 94 per cent. The proportion of dysentery bacilli to other organisms varies a great deal so that greater search is necessary in some cases than in others.

In making the bacteriological diagnosis normally passed stools are preferred for it is from these that mucus, pus or blood can be most easily selected and separated from the fecal material. Slides of the material selected should be made and stained by Gram's method and the usual method for the demonstration of tubercle bacilli. Suspensions of the material selected are then made in tubes of sterile bouillon or salt solution and from these various dilutions are plated on an agar medium. In the different laboratories plain agar, lactose litmus agar, Endo's medium, and so on are preferred for plating². After twenty-four hours of incubation the small, slightly bluish colonies not fermenting lactose are selected and from them tubes of Hiss' semisolid tube medium (page 207) are inoculated by the stab method. Dysentery bacilli do not produce gas on dextrose and are non-motile. Only the cultures showing the proper effect on the semisolid medium are carried further to litmus milk, gelatin and agar slants. All of the organisms producing only slight acidity on milk and not liquefying gelatin are then tested for by agglutination tests with specific antidysenteric serum. Only those cultures having the right characteristics and agglutinating in 1:50 or greater dilutions of the serum are to be regarded as dysentery bacilli. To differentiate between the different types of dysentery bacilli observations are made on the effects of inoculation of Hiss' serum water containing the various carbohydrates. The

¹Bacteriological and Clinical Studies of the Diarrheal Diseases of Infancy, 1904.

²Kendall and Walker: J. Med. Research, 1910, XXIII, 481.

organisms may be isolated from the stool, mucous membrane and intestinal ulcers during the disease. They do not invade the blood stream so that blood cultures are negative.

SERUM REACTIONS.—Dysentery bacilli produce specific and common agglutinins as a result of infection and on injection of living or dead bacilli into man and the animals. The other organisms of the typhoid-colon group also produce agglutinins for dysentery bacilli so that agglutination of dysentery bacilli with blood from normal adults frequently occurs in dilutions of 1:20 or 1:30.

Knox and Schorer found that the blood in dilutions of 1:20 or 1:50 agglutinated the Flexner-Harris type in 51.5 per cent. of the cases from which dysentery bacilli were isolated and the dysentery organisms isolated from the patient in 65.7 per cent. of the cases. Of 18 cases the blood from which did not agglutinate the Flexner-Harris type, 14 yielded isolations of *Bact. dysenteriae*. Flexner concludes that the blood of the children suffering from diarrhoeal diseases at times agglutinates the bacillus of dysentery in high dilutions but this agglutination does not proceed hand in hand with occurrence of the bacillus in the intestine. Richards, Peabody and Canavan¹, in dysentery among the insane at Danvers found positive agglutination in one of four cases tested on the first day of symptoms, in 52.6 per cent. of all cases tested during the first week, in 92.3 per cent. during the second week and in 100 per cent. of the cases tested during the third and fourth week. In this connection it must be mentioned that they found the Shiga type of bacillus the prevailing type in their epidemic while among children the mannite fermenting types are most frequently found. In children's dysenteries agglutination tests are certainly of little diagnostic value as far as evidence of infection with or presence of dysentery bacilli is concerned. Other serum tests have been advocated but have not been found to possess greater value than the agglutination test.

Immunity and Specific Therapy.—Immunity of man to infection with dysentery bacilli is still unsolved. Apparently there is some degree of natural immunity. Several repeated attacks of dysentery within a few years do not indicate that immunity is not acquired by one attack because all of the attacks may be due to the same infection which has become chronic. Experiments in animals

¹Richards, Peabody and Canavan: Boston Med. and Surg. Jour., 1909, CLXI, 687.

indicate that some considerable degree of immunity may be actively acquired. In man and the animals sera containing rather large amounts of antibody are produced.

Vaccine Therapy and Active Immunization.—Shiga in 1898 tried active immunization by subcutaneous injections of small amounts of killed cultures of the Shiga type of *Bact. dysenteriae*. These injections produced such marked reactions that he later tried injections of antidysenteric serum and killed cultures simultaneously and followed these three to five days later by injections of larger doses of vaccine alone. By this method the local reactions were less severe. Dopter to avoid reactions used sensitized dysentery bacilli of the Shiga type. His method is to kill the organisms by heating to 60° C. for one hour, dry them in a vacuum, suspend them in physiological salt solution and then add a sufficient amount of potent antidysenteric serum to agglutinate the bacilla so they fall to the bottom. The precipitate he washes with salt solution and then injects it subcutaneously. Injection of sensitized killed bacilli he found produces no reaction but a considerable immunity. Lucas and Amoss¹ have tried immunization by the combined method of Shiga for prophylactic and curative purposes in the dysenteries of children, but instead of immunizing to the Shiga type of the bacillus used the Flexner-Harris type. Their results are inconclusive.

Active immunization for curative or protective purposes by any of the methods is still unsatisfactory. In 10,000 people vaccinated by Shiga he noted a decrease in mortality but no decrease in the number of cases or severity of symptoms. In active immunization either sensitized bacilli should be used or else the first injection of vaccine should be accompanied by antidysenteric serum and the difference in types of dysentery bacilli should be borne in mind. The duration of experimentally acquired immunity is about two months.

Serum Therapy and Passive Immunization.—Soon after the discovery of the bacillus of dysentery by Shiga in 1892 the treatment of bacillary dysentery by the use of immune sera was undertaken. It was found by Shiga that the organism which is now regarded as the etiological factor in bacillary dysentery is readily

¹Lucas and Amoss: Jour. Exper. Med., 1911, XIII, 486.

agglutinated with blood from patients suffering with this disease. Following the discovery of the etiological importance of this organism in the dysenteries in Japan similar observations were made in various parts of the world. At first these organisms were all regarded as being alike but soon were found to belong to one or the other of two main types: one type (Shiga) not fermenting mannite and producing an extracellular toxin, and a second type (Flexner-Harris, Hiss Y and so on) fermenting mannite and not producing an extracellular toxin.

Because of the differences in ability to produce extracellular toxin, antitoxic immunity can be produced by experimental immunization for the Shiga type while for the mannite fermenting types the production of antitoxic immunity is not possible experimentally. These differences were not recognized before much work had been done on the treatment of bacillary dysentery with specific immune sera. Because of this the value of serum therapy in dysentery can be determined only from the relatively recent literature on this subject.

With knowledge of the essential differences in the types of dysentery bacilli the problem of serum therapy has been more scientifically studied but still the difficulties have not been entirely overcome. That a true antitoxic serum can be made for the Shiga type of bacillus does not solve our problem because with us by far the largest number of cases are associated with infection by the mannite-fermenting types and for these there is no antitoxic serum. The method of preparing the specific antiserum for the Shiga type consists in first injecting the horse with antidysenteric serum and on the same or next day injecting a small dose of toxin or toxin culture. The amount of toxin is increased at each injection, the interval between injections being about five days. Usually killed or living bacilli are also injected to make the serum bactericidal as well as antitoxic. Immunization of animals to the Shiga type is difficult as many of the animals die during the process. Preparation of antiserum for the mannite-fermenting types is not as severe on the animals. Injections of antiserum and killed dysentery bacillus cultures are first made and then followed by increasing doses of dysentery bacilli, both killed and living. This serum is agglutinating and bacteriolytic but not antitoxic.

The Shiga antiserum is standardized by injections of serum and toxin into mice or rabbits. The serum should be potent enough so that 0.5 c. c. will protect a 1500-gram rabbit against three times the smallest fatal dose of the toxin. The antiserum to the mannite-fermenting types is not standardized to any degree of accuracy.

The results obtained by the use of antidysenteric serum in the treatment of dysentery have differed markedly. In Japan by the use of Shiga's antidysenteric serum the mortality of dysentery has been reduced from 22 to 26 per cent. to 9 to 12 per cent. Vaillard and Dopter have collected statistics on two hundred cases treated with antidysenteric serum in which cases there was a mortality of only 2 per cent. In the United States on the other hand no great beneficial results have been attained by the use of the serum as is evidenced from the extensive investigations made in 1903 under the direction of Dr. Flexner. There are various reasons for the differences in results which have been obtained by the use of antidysenteric serum. The patients treated in the United States are largely children under three years of age while a large percentage of the cases for which favorable results are obtained with serum treatment occur in adults. The day of the disease on which serum injections are made is probably earlier in such locations where epidemic dysentery is regarded as a serious disease. In the United States many cases of frequency of stool are only simple diarrhoeas and because of this the patient usually receives no medical attention until the disease is well advanced. Probably the most important reason for the difference of curative values of the different sera is dependent on the differences in the specific substances in the serum. Evidently those sera which have produced the most beneficial results possess antitoxic properties while those used in the United States are principally or wholly antibacterial. Shiga says of his serum that it is "bactericidal as well as antitoxic and therefore is more effective than antityphoid serum." Practically all of the antidysenteric sera have been polyvalent, i. e., the animals furnishing the serum have been immunized to the various strains and toxins of dysentery bacilli.

The method of treatment with antidysenteric serum varies with the severity of the disease. Shiga suggests that in mild cases one dose of 10 c. c. of the serum be injected. In cases of medium

severity two injections of 10 c. c. each, the interval between injections ranging from six to ten hours, are recommended. In the severer cases 40 to 60 c. c. in all are to be injected but never more than 20 c. c. daily. The serum used in the United States has been injected in larger amounts, sometimes as much as 100 c. c. being injected in one day.

The effect of treatment with Shiga's serum has been to decrease the number of stools, cause the blood and pus to disappear from the stools, restore the temperature to normal and to lessen the pain and tenesmus. When this serum is used late in the disease the beneficial effects manifest themselves more slowly. The serum used in the United States has not influenced the course of the disease to any extent nor is it possible to determine any particular improvement in the condition of patients so treated.

Coyne and Auché have reported eleven cases of dysentery produced by the Flexner or mannite-fermenting type of dysentery bacillus treated very successfully with a polyvalent serum. The curative effects of this serum were probably due to the action of the antitoxin to the Shiga type of bacillus dysenteriae.

It is to be hoped that specific antitoxic sera can be made for the mannite-fermenting group of dysentery bacilli or that Shiga type antitoxin will be found to be efficient in combating infections with the mannite-fermenting group of dysentery bacilli. This is especially desirable because the mannite-fermenting organisms are largely responsible for the children's summer diarrhoeas in the United States.

Prophylaxis.—The general problems of prophylaxis against bacillary dysentery are similar to those met with in typhoid fever. Dysentery bacilli of the mannite-fermenting type have been found in the digestive tract of normal individuals and may only be waiting for some insult, as error in diet and so on, to allow them less restrained growth. In children undoubtedly errors in diet are of importance. It cannot be accepted that all of the cases of children's dysenteries are due to infected milk for the disease occurs at times in breast-fed children and in many of the cases is ushered in by improperly constituted diet. It has been held by some that in the adult only the true Shiga type is a parasite. Protective inoculation, vaccination and injection of antidysenteric serum gives an immunity

of only short duration and with us except in institutions seems wholly out of the question at this time.

INFECTIONS WITH *BACILLUS COLI COMMUNIS*.

Bacillus coli is very widely distributed in nature and is a common inhabitant of the digestive tract in man. While usually the organism causes no ill effects it at times is the cause of various pathological conditions and enters into infections with other bacteria. Colon bacilli may leave the digestive tract, enter the blood stream and produce disease in organs ordinarily free from bacteria. This may occur with or without injury to the intestinal canal. To cause disease especially virulent types are not necessary, the severity of the disease in most cases depending on reduced resisting powers or else mechanical irritation. Colon bacilli belong to the group that has already been referred to as the typhoid-colon group. There are many varieties among the species as is determined by fermentation and agglutination tests.

Differential Specific Diagnosis.—CLINICAL.—Colon bacillus infections except in peritonitis run a slow course, producing a low grade of fever and some pus. They locate especially in parts of the body where the fluids exert little bactericidal power as in the genito-urinary tract, and bile receptacles and passages. The organisms are the usual cause of peritonitis in intestinal perforation but may also cause peritonitis if the intestinal wall remains intact. Peritonitis produced by colon bacilli may remain localized or be diffuse, may be acute or chronic and usually considerable odor is produced. Wounds infected with the organisms usually show evidence of much destruction.

BACTERIOLOGICAL DIAGNOSIS.—Isolation of *Bac. coli* from the stools means but little as the organism is a common inhabitant of the intestinal tract. From the diagnosis of colon bacillus infection bacteriological examination is necessary. This cannot be made from stained slides alone. Isolation of pure cultures, determination of cultural and staining characteristics, motility and morphology are absolutely essential to make the diagnosis of *Bac. coli* infections. The organism is a motile bacillus, does not hold the Gram stain, produces acid and gas in most of the carbohydrate media, does not liquefy gelatin and coagulates milk. For further points in differ-

entiation from other organisms the text books on bacteriology are referred to.

SERUM DIAGNOSIS.—*Bac. coli* produces agglutinins just as do the other organisms of this group. The blood of normal persons and that of patients having diseased conditions not caused by *Bac. coli* may agglutinate the organisms. This may be due to former or present infection with any of the organisms of this group.

Immunity and Specific Therapy.—Immunity to *Bac. coli* is slight. The acute infections are seldom treated by vaccines and sera. Different infections produced by *Bac. coli* however have been treated by injections of vaccines made with this organism. The vaccine method of treatment has been found to be most successful in cases of chronic cystitis due to *B. coli communis*, although cholecystitis, appendix abscesses, endometritis and other local infections with this organism have at times responded favorably to this method of treatment. The number of bacteria injected is seldom more than fifty million, usually smaller doses than this being used. Vaccines made from cultures isolated from the patient usually give the best results. The injections are repeated about every ten days.

INFECTIONS WITH VIBRION CHOLERÆ.

Asiatic cholera first came from the delta of the Ganges River. The disease first existed pandemically in Europe in the beginning of the nineteenth century and first occurred in the United States in 1832. While there have been several epidemics in this country the disease is of importance to us only as travelers come from cholera-infected countries abroad. *Vibrio cholerae* was discovered by Koch in 1883, the organisms being isolated from the stools of cholera patients.

Differential Specific Diagnosis.—**CLINICAL.**—Clinical diagnosis is easy when epidemics exist. The disease is characterized by violent purging, tenesmus, vomiting, loss of water from the tissues, cyanosis and collapse. In these respects it differs little from our *cholera nostras*, to distinguish it from which bacteriological diagnosis is necessary.

BACTERIOLOGICAL DIAGNOSIS.—Because of the acuteness of the illness and seriousness of epidemics, the bacteriological diagnosis is divided into two parts: the tentative and the absolute. Mate-

rial for diagnosis should be obtained from the stool or scraping of the intestinal mucosa. Blood and urine cultures are of no value as they do not contain the organisms. From the feces or scraping, mucus and flecks of tissue are placed on slides, fixed and stained by dilute carbol-fuchsin and if there is a predominance of typical comma-shaped organisms an early tentative diagnosis can be made. Dunham's medium of peptone should however also be inoculated and incubated at 37° C. Cholera organisms multiply rapidly and grow aerobically so that at the end of six to ten hours they will have multiplied sufficiently that almost pure cultures can be demonstrated in stained preparations from the upper part of the peptone water medium. Further inoculations are made for this material in alkaline agar plates, gelatin, Dunham's solution, and so on. The organisms produce in Dunham's solution the so-called cholera-red reaction. This is due to the formation of nitroso-indol, which is demonstrated by the addition of a few drops of sulphuric or hydrochloric acid to a 48-hour culture in Dunham's solution. This reaction has only a negative value. When it is not produced cholera vibrios can be excluded. To make the final diagnosis agglutination and lysis tests should be made with anticholera serum.

SERUM DIAGNOSIS.—The blood of patients having had cholera agglutinates cholera vibrios but for diagnostic purposes serum tests are of little value as the reactions only occur late in the disease. Tests with blood from animals immunized artificially are of value in the identification of the organisms.

Immunity and Specific Therapy.—Not all persons are equally susceptible to the disease. The acidity of the gastric juice offers a barrier to entrance of living cholera vibrios into the intestines.

In the disease and during experimental immunization agglutinins, precipitins, lysins and opsonins are produced. The duration of these antibodies is relatively short. While intoxication during the disease is marked soluble toxins are not produced in the culture media. The toxic substances are closely bound to the organisms and only liberated when the bacilli disintegrate. Dorr, Kraus and others believe they have obtained a true soluble toxin but this has not received much confirmation.

Vaccine Therapy and Active Immunization.—In 1884 Ferran

tried prophylactic immunization but apparently he was not working with pure cultures. Haffkine tried active immunization for prophylactic purposes on a large scale in India. Up to 1895 he had vaccinated some 40,000 persons by a method based on the procedure of Pasteur in anthrax, hydrophobia and so on. He injected first a vaccine of living cholera organisms attenuated by prolonged growth at 39° C., and five days later introduced hypodermically living virulent cultures. His results while not conclusive showed some degree of protection. Kolle, finding that bacteriolysins are the protective substances produced in man during the disease and that these can be produced by injections of killed as well as living cultures, injected 2 m. g. of culture in 1 c. c. of salt solution. The cultures used were killed by exposure at 58° C. for one hour. For the second injection he uses 4 m. g. of culture. The results of this method in the Japanese Army are certainly indicative of protective value for out of 77,907 persons vaccinated 0.06 per cent. had the disease and 0.02 per cent. died, while of 825,287 persons not vaccinated the morbidity was 0.13 per cent. and the mortality 0.10 per cent. From this it will be seen that the disease in the vaccinated was milder than in the unvaccinated. The protection is of short duration and active immunization for curative purposes is of no value.

Serum Therapy and Passive Immunization.—Serum therapy has been of little value. Because of the intimate association of the toxin with the organisms (endotoxin) only antibacterial sera can be produced in animals. The sera of Roux, v. Behring, Metchnikoff and others, while of value in animals when injected before or with cholera vibrios, have no curative value.

Prophylaxis.—Infection occurs in the digestive tract. To prevent this infection contamination of food must be avoided. In times of epidemics the stools of patients should be examined at once, cholera patients should be isolated, the stools thoroughly disinfected, carriers detected and isolated and all efforts made to keep the organisms from the water supply. Prophylactic immunization is recommended for doctors and nurses coming in contact with cholera patients and in cases of epidemics it is advisable to immunize whole communities. It is to be remembered that this immunity is of short duration.

INFECTIONS WITH BACILLUS PESTIS.

Bubonic plague under the name of "black death" in the fourteenth century was responsible for approximately 25,000,000 deaths in Europe. In the United States the disease has never assumed serious proportions. The causal factor of the disease was discovered in 1894 by Kitasato and by Yersin.

Differential Specific Diagnosis.—CLINICAL.—Two types of the disease are recognized, the ambulatory and the severe. In nearly all cases there are glandular swellings or buboes which may or may not break down. Early in the disease the effects of intoxication become evident, the pulse being irregular and thready, there is fever, giddiness and delirium. The best diagnosis is based on the bacteriological findings.

BACTERIOLOGICAL DIAGNOSIS.—For bacteriological examination material is obtained by puncture of the bubo with the hypodermic needle or from the circulating blood. The organisms may also at times be obtained from the sputum. With this material slightly alkaline bouillon is inoculated and a number of guinea-pigs or rats are given subcutaneous injections. *Bac. pestis* is a non-motile bacillus, not holding Gram's stain, and polar bodies can be demonstrated with methylene blue. When grown in bouillon chains are formed. The organisms are agglutinated with antiplague serum. In guinea-pigs and rats subcutaneous injections cause death in a few days. In these animals the glands and spleen are enlarged. Lesions are also found in the other organs.

SERUM DIAGNOSIS.—In the disease agglutinins usually occur in the blood while there are none in the serum of healthy individuals. The agglutinins however do not appear early enough to be of value in the diagnosis. The reaction is of value in the identification of plague bacilli.

Immunity and Specific Therapy.—An attack of the disease confers immunity for a number of years. The organisms do not produce a soluble toxin. In experimental immunization it has been found that injections of killed cultures are not as efficient as living attenuated cultures.

Active Immunization and Vaccine Therapy.—Haffkine advocated active immunization for prophylactic purposes. The vac-

cine is prepared from a four to six weeks' bouillon culture incubated at 25 to 30° C. This is heated to 65° C. for one hour. Then he adds 0.5 per cent. phenol. Adults at first receive an injection of from 2.0 to 3.5 c. c. and ten days later a larger injection, the doses depending on the reaction following the first injection. Lustig and Galeotti have extracted the bacilli with weak potassium hydrate and injected from 2 to 3 m. g. of the dried powder. Kolle and Strong have immunized with living cultures of *Bac. pestis* so attenuated that two agar cultures do not kill a guinea-pig. The results of active immunization for prophylactic purposes have been of value. These methods have not been advocated for curative purposes.

Serum Therapy and Passive Immunization.—Animals receiving first injections of killed and later of living cultures of *Bac. pestis* develop agglutinins, bacteriolysins and probably also opsonins. In a number of laboratories horses have been immunized in this way and antiplague serum obtained. Lustig and Markl have injected "toxins" into animals and claim to get antiserum that is not only bactericidal but also antitoxic. The different sera have apparently protective and curative value but the duration of protection is only for three to four weeks. It is probably advisable to administer combined vaccines and sera to persons that are exposed. If beneficial results are to be obtained from antiplague serum in the treatment of the disease the injections must be made early. The first injections are usually made intravenously and the subsequent ones subcutaneously. Reports on the curative value of antiplague serum vary greatly.

Prophylaxis.—Plague has so far caused us little anxiety but inasmuch as the disease exists in various parts of the world at all times and has prevailed in some of our possessions protective measures are of momentous import to us at all times. The great protective measures center about the early diagnosis of infection in man and in rats and other rodents. Efficient quarantine and detention of immigrants from infected ports, extermination of rats, destruction of houses where rats exist and the construction of rat-proof buildings are of great importance. Protective immunization of persons exposed or likely to be exposed is advisable whenever the disease is introduced.

INFECTIONS WITH BACT. DIPHTHERIÆ.

Diphtheria is an acute communicable disease caused by the *Bact. diphtheriæ* of Klebs and Loeffler. Differentiation of this disease from other conditions was made by Bretonneau and by Trousseau. It was not however until the bacillus of diphtheria was described by Klebs in 1883 and cultivated by Loeffler in 1884 that the disease could be definitely recognized. Infection with the organisms produces local lesions especially in the pharynx, larynx, trachea, nose and lungs, and is accompanied by general lesions and symptoms of varying degrees of severity. Infections of the blood stream are rare. Parenchymatous organs, lymphatic glands and the cells of the nervous system have receptors for the diphtheria toxin. To all infections produced by *Bact. diphtheriæ* the term diphtheria should be applied. Under certain other conditions not associated with the diphtheriæ bacillus a similar local lesion referred to as a diphtheritic membrane may be formed. Diphtheria may be of varying severity. In a considerable number of cases diphtheria bacilli are present for long periods after recovery from the disease or even occur in persons never having had the disease. Persons no longer sick but carrying the bacilli and patients having recovered and still harboring the organisms are referred to as bacillus carriers.

Differential Specific Diagnosis.—CLINICAL.—Diphtheria is usually manifested by a membrane especially in the mucous membrane of the tonsils, soft palate, uvula, pharynx, nose, trachea and bronchi, and at times on the mouth, lips, esophagus, conjunctiva, middle ear, stomach and genitalia. The membrane usually appears first on the tonsil, may involve only one tonsil or may extend to both after 24 hours.

The membrane varies in appearance, in most cases is grey or greyish yellow but may be white, yellow, green or even black. It consists of fibrin cells and granular material and depending upon the proportion of these may be firm, tenacious, soft, pliable or granular. On removal of the membrane usually a bleeding surface is left. A distinguishing character of the membrane is its tendency to spread and extend. In some cases there is only little membrane and in some there is none. When the other symptoms of diphtheria are present and no membrane is seen most careful search should be made in the nose and trachea.

The onset of the disease varies but a blood-tinged nasal discharge, symptoms of croup early in the disease, enlargement of the cervical lymph glands, chilliness, fever, aching pains in the back and limbs, and temperature of 102 to 103° F. all point toward diphtheria. If there is a membrane added to these conditions the physician is warranted in making a tentative diagnosis of diphtheria until a positive bacteriological diagnosis can be made. In many cases that have come to my notice diphtheria has been diagnosed as spasmodic croup. This seems unwarranted because diphtheria occurs more frequently than croup, because a throat examination will usually show the membrane or at least some local lesion and because bacteriological examination furnishes the diagnosis.

Clinically different forms of diphtheria are recognized: (1) a purely local infection; (2) a local infection with general intoxication; (3) a septic, malignant diphtheria usually complicated by streptococcus infection. Septic or malignant diphtheria is the most severe form of the disease. Laryngeal and tracheal diphtheria must also always be regarded as very serious. In this condition the voice is hoarse, there is a gradual increase in dyspnoea, the constitutional symptoms are not so severe, the temperature ranges from 99 to 101° F., symptoms gradually increase, respirations become very noisy and difficult, cyanosis comes on and the temperature rises until shortly before death it may reach 105 to 106° F. Death frequently occurs within 24 hours after the appearance of the first symptoms.

BACTERIOLOGICAL DIAGNOSIS.—Bacteriological methods offer the safest means of diagnosis. The diphtheriæ bacillus often referred to as the Klebs-Loeffler bacillus is a small, slightly curved, non-motile organism. It occurs in many forms and is especially likely to produce involution forms that are club-shaped or may appear as being branched. In a swab from the throat they often are V or Y shaped. The organism holds the Gram stain. For differential diagnosis Loeffler's methylene blue, dilute Ziehl's carbol-fuchsin and Roux's stain are most valuable for with them the so-called Ernst-Babes bodies are brought out in direct smear preparations and in cultures nine to twenty-four hours old. These appear as darkly stained bodies in the faintly stained protoplasm of the bacterial cell.

For diagnostic purposes smears and cultures are usually made. For cultivation Loeffler's blood serum medium is best as the diphtheria bacilli grow rapidly on this medium while other bacteria are inhibited for a time at least. On this medium *Bact. diphtheriae* produces small, white, discrete colonies, similar to those produced by streptococci on most media. Stained preparations made from cultures 9 to 24 hours old usually show many diphtheria organisms as identified by the involution forms and bacilli containing the Babes-Ernst granules. For details of the cultural characteristics the reader is referred to the text books on bacteriology.

Material for microscopical and bacteriological examinations is usually obtained from the local lesions in the nose and throat. For this purpose swabs on a wood or metal applicator are used. After winding a small amount of absorbent cotton on one end of the applicator it is put into a test tube and sterilized in the oven. To obtain the material the patient should be placed in a position as for a throat examination so that good light and exposure of the lesion are obtained. This cannot be too strongly emphasized. There can be no doubt that unsatisfactorily made swabs are responsible for negative cultures and smears in many cases of diphtheria. With the patient in good position and the free end of the sterile swab held tightly by the examiner, the cotton-covered end is firmly but gently rubbed over the membrane, or if there is no membrane over the inflamed mucous surface. Then without putting the applicator down or turning it, the surfaces of several tubes of Loeffler's blood serum are inoculated with that surface of the swab that has been rubbed over the lesions. After this smears should be made and stained. The stained preparations are then examined and if club, V or Y shaped bacilli are found a tentative positive diagnosis of diphtheria should be made. If there are granular bodies the diagnosis is more positive. On these findings antitoxin injection into the patient is warranted. Before further steps are taken the cultures made should be examined and if stained preparations of nine to twenty-four hour growths show organisms with the right staining and morphological characteristics, the physician should institute quarantine and such other measures as are necessary.

Cultures have also been made of the blood but as diphtheria usually is not essentially a blood-stream infection blood cul-

tures are usually negative. Conradi and Bierast found diphtheria bacilli in the urine of 54 out of 155 diphtheria patients, but this is too small a percentage to be an aid in diagnosis.

Unfortunately diphtheria bacilli are distinguished with difficulty from other organisms called pseudodiphtheria bacilli. These include *Bact. xerosis*, the bacillus of Hoffman, the bacillus of Ruediger, and so on. These organisms are usually shorter, grow more luxuriantly on ordinary media, and while diphtheria bacilli produce acid on dextrose and levulose the pseudodiphtheria bacilli do not. For the final differential diagnosis of pseudodiphtheria organisms from diphtheria bacilli injections of animals with cultures and toxins are necessary. Fortunately pseudodiphtheria bacilli are seldom present in throats of patients where diphtheria is suspected. Kolmer from complement fixation tests believes that the organisms are related.

Serum Tests are little used in diagnosis. In artificial immunization antitoxins, complement fixation bodies, and so on are produced.

Immunity and Specific Therapy.—The immunity conferred by an attack of the disease lasts only for one to two months. In no disease however has work on induced immunity been of as much value as in diphtheria.

Ferran early in 1890 and Fraenckel and Behring later in the same year published methods by which experimental animals can be immunized to diphtheria. Behring and Kitasato in 1891 published methods for the immunization of guinea-pigs to diphtheria toxin. In 1892 Behring and Wernicke emphasized the presence of protective substances in serum of diphtheria-immune animals. Serum therapy as applied to the treatment of diphtheria in man began in 1891 and 1892, and in August 1894 diphtheria antitoxin was put on the market. Since then it has been used extensively.

The organism produces an extracellular toxin so that real antitoxic immunization is possible.

Active Immunization and Vaccine Therapy.—Active immunization of horses is employed to obtain diphtheria antitoxin. The antitoxin methods for treatment and protection against infection have been so successful that active immunization has

not been used to any great extent. Recently Hewlett and Nankivell¹ and Petruschky² have tried it for the purpose of freeing bacillus carriers from diphtheria bacilli. To prepare the vaccine solid culture media is inoculated, the culture incubated and the growth washed off. Hewlett and Nankivell grind up the bacteria and prepare a germ-free filtrate, while Petruschky kills the organisms with chloroform. Their results on destroying the bacteria in carriers are good and it may be found that these vaccines may be used to confer a protective immunity. Active immunization undoubtedly cannot replace serum therapy in the treatment of the disease.

Passive Immunization and Serum Therapy.—Treatment of and protection against diphtheria by means of diphtheria antitoxin has been practiced since 1894.

The method of preparing diphtheria antitoxin has gone through various stages of development. These it is not necessary to state as now practically all antitoxin used in the United States is made after one method. The animal chosen for elaboration of diphtheria antitoxin is usually the horse. This is first tested by tuberculin and mallein injections to make certain that it is free from tuberculosis and glanders. It is examined physically and then given an immunizing dose of tetanus antitoxin to protect against accidental infection with tetanus.

The toxin used for active immunization of the horse is now obtained the world over from a strain of the diphtheria bacillus especially studied by Park and Williams of the Research Laboratories of the New York Health Department. This culture is grown in two per cent. bouillon of an alkalinity of 0.8 per cent. above the neutral to litmus. A small amount of the bouillon is placed in Ehrlenmeyer flasks and inoculated with the diphtheria bacilli. After one week's growth at 37° C. the culture is tested for purity, the organisms are killed by adding 10 per cent. of a 5 per cent. phenol solution and allowing the same to act for forty-eight hours. The clear supernatant fluid is then drawn off, filtered through a Berkefeld filter and tested

¹Hewlett and Nankivell: *Lancet* (London), 1912, CXXXXIII, 143.

²Petruschky: *Deutsche med. Wehnschr.*, 1912, XXXVIII, 1319.

for potency. If 0.01 c. c. of the filtrate kills a 250-gram guinea-pig on or before the fourth day it is considered suitable for purposes of immunization.

The horse to be immunized receives enough of this toxin in bouillon to kill five thousand guinea-pigs of 250 grams each. At the time this amount of toxin is injected, the horse also receives an injection of ten thousand units of diphtheria antitoxin. After three to five days when the fever has subsided a somewhat larger dose of toxin and the same amount of antitoxin are injected. A third injection is made after another interval of from three to five days. After this usually no more antitoxin is injected with the toxin, the doses of which are constantly increased and injected at intervals of from five to eight days. After about two months of treatment if the immunization has been successful the horse will usually tolerate enough toxin at one injection to kill one hundred thousand guinea-pigs of 250 grams each. At the end of six weeks to two months samples of blood are drawn and tested for protective value. If the antitoxic value is high (from 200 to 600 units per c. c.) the horse is bled by tapping the jugular vein. The withdrawal of serum is made under aseptic conditions. The bottles containing the blood are slanted and after four to five days the serum is drawn off. The amount of blood drawn off varies but usually about 5,000 c. c. are taken at intervals of one month. One horse can only furnish about 35 liters during the year as periods of rest are necessary.

By means of Gibson's or Banzhaf's method the serum is now concentrated and refined (page 122). After this it is standardized, i. e., its strength is determined. The United States government determines the unit of diphtheria antitoxin in the United States and the Government requires that all diphtheria antitoxin made by manufacturers having a United States Government license must conform to this standard. This emphasizes the importance of using only diphtheria antitoxin made by manufacturers having a United States Government license. Because the toxic value of diphtheria toxin changes with ageing and other conditions, the Hygienic Laboratory of the U. S. Public Health Service issues from time to time standard anti-

toxic serum. With this serum the strength of the toxin used in determining the antitoxic value of serum is gauged. The "immunity unit" or unit of antitoxin is contained in the amount of diphtheria antitoxic serum which will just neutralize one hundred times the smallest amount of toxin necessary to kill a 250-gram guinea-pig in four days. The antitoxic value of serum is determined by mixing one hundred times the smallest fatal dose of fresh diphtheria toxin with varying amounts of the diphtheria antitoxin, allowing the mixtures to stand for fifteen minutes and then injecting them into suitable guinea-pigs. The smallest amount of serum which will protect a 250-gram guinea-pig for more than four days against one hundred times the smallest amount of diphtheria toxin necessary to kill a 250-gram guinea-pig contains one unit of diphtheria antitoxin.

At the time of bleeding immunized horses, that is after six to eight weeks of immunization, the serum may contain from one hundred to one thousand units of diphtheria antitoxin per c. c. The concentrated and refined diphtheria antitoxin contains usually from three hundred to two thousand units of antitoxin per c. c. The various manufacturers of diphtheria antitoxin now furnish their product in syringes containing a determined number of units of antitoxin. These packages are stamped so as to indicate the dates after which they can be exchanged free of charge for more recently tested serum. Because of the degeneration in potency of diphtheria antitoxin in the fluid condition, it is desirable that no sera be used after the date when they are to be exchanged for new sera.

The injections of diphtheria antitoxin are usually made into the subcutaneous tissue on the abdominal wall or between the shoulder blades. Intramuscular injections have also been made. More recently intravenous injections have been advocated. Berghans has found that on injection of the same amount of diphtheria toxin into different guinea-pigs, five hundred times as much antitoxin is necessary to save the pig by subcutaneous injections than by those made intravenously.

It has also been observed that the amount of antitoxin in the circulating blood does not reach its maximum until after two

to three days if it is injected into the subcutaneous tissue. Park¹ has determined that a few minutes after subcutaneous injection of diphtheria antitoxin the blood-stream becomes feebly antitoxic, but after several hours becomes strongly so. Inasmuch as antitoxin neutralizes any toxin in the blood as soon as the antitoxin enters the blood stream, Park advocates intravenous administrations. In this way passage of toxin from the blood stream is stopped and further injury to the cells prevented. Park² has shown that suitable subcutaneous injections of antitoxin in six hours yield 2 units per c. c. of blood while after the same time after intravenous injection there are 20 units. At the end of twenty-four hours intravenous injections yield 12 units and subcutaneous 6 units per c. c. of blood. He feels certain that 5,000 units given intravenously have as much effect as 20,000 units given subcutaneously. Antitoxin injected intravenously leaves the capillaries only slowly so that some time is lost before toxin that has passed out is neutralized.

The **methods of treatment** of diphtheria with diphtheria antitoxin vary. However some definite principles of treatment have been established. It is very essential that all cases be treated as early as possible. The reasons for this are clear when it is understood that as toxin is liberated by diphtheria bacilli it tends to combine with the cells of the body. After this union has once taken place the cell is rapidly injured. In order that diphtheria antitoxin combine with these toxins it is essential that the immune substances be present at the time of the liberation of the toxin. Most statistics show that in ordinary cases of diphtheria the death rate is **nil** when diphtheria antitoxin is injected on the first day of the disease.

The death rate when injections are made on the second day is below five per cent. After the third day of the disease diphtheria antitoxin becomes less effective being approximately 12 per cent. when injected on the third day, 15 per cent. on the fourth and fifth days, and greater than 20 per cent. after the fifth day.

The **dose** to be injected varies and no firm and fast rules can

¹Park: J. Am. M. Ass., 1912, LVIII, 453.

²Park: Boston Med. and Surg. Jour., 1913, CLXVIII, 73.

be laid down. For some time repeated injections of antitoxin were advocated but more recently single injections of large doses have been recommended. Park has reported experiments in which two goats were used. Of these one received one injection of 15,000 units subcutaneously and the second received four doses of 5,000 units at intervals of eight hours. At the end of eighteen hours the first goat contained 12 units per c. c. of blood while in the blood of the second there were only $3\frac{1}{2}$ units per c. c. It was not until after three days that the blood of the second goat contained as much antitoxin as did the one receiving 15,000 units at one injection. Inasmuch as we want the antitoxin early and the antitoxin in itself is harmless Park recommends one injection of large dose.

With this in mind the doses to be recommended are larger than those formerly advocated. In the usual case of diphtheria seen on the first day a child should receive from 5,000 to 10,000 units. When the patient is not seen until the second day 10,000 to 15,000 units should be given. The injections on the first or second days may be made subcutaneously but on and after the third day should be made intravenously. In the pharyngeal or laryngeal types it is best to give intravenous injections especially when the symptoms are severe.

When the disease comes under observation late or is very severe twenty thousand to one hundred thousand units may be necessary. Evidence has been obtained that toxin may be taken from the cells with which it has combined when very large doses of antitoxin are injected. No case of diphtheria should be regarded as being too severe or too far advanced to be treated by diphtheria antitoxin but in such cases large doses should be given, as small doses are of no avail because they do not neutralize all the toxin present. The age of the patient, unless very young, should have no influence on the amount of antitoxin injected.

Diphtheria antitoxin as any other therapeutic agent should be given in sufficient quantities to accomplish the full therapeutic action. To determine this the effects of diphtheria antitoxin must be recognized. The results of injections of sufficient amounts of antitoxin are: general improvement of

the patient's condition, reduction of fever, improvement of the pulse, but most noticeable of all is the shriveling of the membrane, decrease of discharge and less fetid odor of the breath. When these effects do not appear from six to eight hours after injection more diphtheria antitoxin should be injected. Re-injections usually are larger than first injections and are indicated at any time when the patient's condition becomes more grave or when improvement does not come in six to eight hours after injection. At times diphtheria antitoxin does not give satisfactory results in the treatment of diphtheria. In most of these cases the specific treatment is begun after the toxin has produced its damaging results or there is infection with other micro-organisms. Of these organisms streptococci are most important and are responsible for many of the fatal cases of diphtheria. Such cases are at times treated with both diphtheria antitoxin and antistreptococcic serum.

Various attempts have been made to administer diphtheria antitoxin by the mouth. The work of McClintock and King on this method has already been referred to. Before oral administration of diphtheria antitoxin can be generally employed more experimentation will be necessary. In some of the severer cases of nasal diphtheria, antitoxin has been sprayed on the membrane with some beneficial results. Injections of antitoxin into the spinal canal have been advocated to bring the antitoxin into direct contact with the affected nerve cells. The use of dried diphtheria antitoxic globulins which are dissolved in salt solution before injection is relatively recent. If the results obtained by their use are as favorable as has been reported it may be expected that they will be readily taken up by the medical profession. Serum however has the advantage of always being ready for use and not requiring a sterilization process before administration.

Coincident with the antitoxin treatment of diphtheria all of the other methods of value should be used. Intubation and tracheotomy may be necessary if there is suffocation. Rendu has advocated exposure of patients to a temperature of 170° F. for five minutes. The most important thing to be considered besides suffocation however is in regard to the effects of

diphtheria toxin on the nervous system, the heart and kidneys for which it shows an especial selective action. To avoid these complications sufficient fluids and absolute rest are necessary even after apparent recovery. Paralysis of the heart, respiratory organs and voluntary muscles are most grave results of the action of diphtheria toxin.

The definitely beneficial results which have been obtained by the use of diphtheria antitoxin in the treatment of diphtheria ought to be sufficient to convince the practitioner that diphtheria antitoxin should be used in practically all cases of diphtheria. Moreover the practitioner ought to know enough concerning the symptoms and complications of diphtheria and the untoward effects of serum injections to distinguish the conditions dependent upon the disease and those dependent upon the serum injected. It is not unusual for the practitioner to diagnose the transient serum rashes as erysipelas and the edema following serum injections as those due to Bright's disease. Practitioners are undoubtedly largely responsible for the misconceptions of laymen concerning the effects of diphtheria antitoxin injections. As a result of these misconceptions frequently consent to use diphtheria antitoxin when it is definitely indicated cannot be obtained and yearly numbers of children whose lives could undoubtedly have been saved are carried to the grave.

Prophylaxis.—This concerns itself with two problems: prevention of the spread of the disease by diphtheria bacillus carriers and with the immunization of those exposed. Persons exposed to diphtheria may carry the organisms in their throats and still not have the disease and likewise a patient may harbor the bacilli after his recovery. It is imperative to isolate effectively such patients and to remove the bacilli from their throats as soon as possible. The quarantine of patients and families where the disease exists is easy but it is hard at times to determine the duration of such quarantine. Inasmuch as the period of time for which diphtheria bacilli may be carried varies, the safe method is to release the quarantine only after at least two negative cultures have been obtained from the patient and attendants and the house, clothing, and so on

have been thoroughly fumigated. To remove the bacilli many methods have been proposed. Sterilization by gargles and sprays while not efficient in all cases are of benefit. Schiotz¹ found that people having sore throat due to staphylococci seldom have diphtheria and that implanting of staphylococci on the tonsil shortens the period for which diphtheria bacilli persist. Catlin, Scott and Day² terminated an epidemic of institutional diphtheria in this way. Ravenal³ uses a spray of staphylococci and believes it important in disposing of the diphtheria bacillus carrier. Diphtheria vaccine has already been referred to (page 234). Any or all of the methods should be used when persistent carriers are met with because carriers are undoubtedly great factors in the spread of the disease.

Immunization of well individuals against possible diphtheria infection has been practiced for some time. It has been quite definitely proven that injection of a relatively small number of antitoxic units will protect from four to six weeks against infection with the diphtheria bacillus. The doses generally recommended are from three hundred to five hundred units in small children and one thousand units for older children and adults. While the custom concerning the immunization of well persons varies, it is quite generally accepted that children who have been exposed to diphtheria should receive at once immunizing doses of diphtheria while adults seldom are treated in this way (see page 133).

INFECTIONS WITH BACT. TETANI.

Tetanus or lockjaw is an infectious disease characterized by tonic spasm of the muscles. In 1884 two Italian investigators Carle and Rattone, succeeded in producing the clinical picture of tetanus in rabbits by injections of material from a case of tetanus in the human. The next year Nicolair observed the tetanus bacillus which was first cultivated in 1887 by Kitasato while working in Koch's laboratory. The organism is widely distributed in nature. *Bact. tetani* is a normal inhabitant of

¹Schiotz: Ugeskrift for Laeger, Dec. 9, 1909.

²Catlin, Scott and Day: J. Am. M. Ass., 1911, LVII, 1452.

³Ravenal: J. Am. M. Ass., 1912, LIX, 690.

the intestinal tract of horses and cattle and at times of dogs, man and so on. It is found quite generally in street manure, and in the soil of fields, barnyards and gardens; in fact in all soils containing feces of horses or cattle as the result of grazing or fertilization. It is not found in forests and places not contaminated by feces. The organism has spores of great resistance which are of much importance in the spread of the disease.

Differential Specific Diagnosis.—Infection occurs principally through wounds. In order that the disease may be produced special conditions must exist. Because it is a strict anærobe the bacillus must usually be deeply imbedded in the tissues. The tissues must be damaged as necrotic tissue favors the development of these bacteria. Foreign material as dirt, splinters of wood and so on in wounds help to produce conditions favorable to the development of tetanus. Saprophytic bacteria favor the development of tetanus possibly because they protect the tetanus organisms from phagocytosis. The incubation period in man varies from two to three days to two weeks but usually is about ten days.

CLINICAL DIFFERENTIAL DIAGNOSIS.—In the diagnosis the cause of the wound in which infection develops is of importance. Penetrating wounds produced by nails and lacerated wounds produced by toy pistols, fireworks, gunshot explosions and so on are most likely to lead to tetanus infection. Wounds contaminated by garden, street or barnyard soil should always be suspected. The first symptoms of the disease to appear are called prodromal symptoms and are headache, lassitude and chilliness. The actual symptoms begin with stiffness of the neck, tightness of the jaw or difficulty in mastication. These are followed by a tonic spasm of the jaw and later of all the muscles. The mouth cannot be opened, the forehead becomes wrinkled and the eyes stare. The entire body may become rigid. In addition to the tonic spasm there are paroxysms of varying duration. In these there is pain, the patient is unable to speak and has profuse perspiration. Some of the untreated cases gradually get well but approximately 90 per cent. terminate fatally. The conditions observed may at times be con-

fused with strychnin poisoning but in this the jaw muscles are not especially involved.

BACTERIOLOGICAL DIAGNOSIS.—This may be difficult. At the time symptoms appear the wound is frequently healed or may never have been observed. When there is a wound, smears and direct cultures may solve the diagnosis. At times however the organisms cannot be obtained in this way. It is usually best to inoculate mice with the suspected material. In this event it is advisable to introduce with the material a piece of sterile thread, wood or glass splinter. From these animals smears and cultures may be obtained. The organism is a slender bacillus holding Gram's stain and forms typical club-shaped spores. It is a strict anærobe. For further bacteriological characteristics the text-books are referred to.

As has already been stated it may be difficult to find the lesion where infection has secured. For a time so-called idiopathic tetanus was referred to. Now it has been suggested that in some of these cases infection may occur in the lungs. Infection at times occurs during childbirth, the mother or child or both being infected. Contaminated antisera have been held responsible for a number of tetanus infections and in making vaccines and sera this should always be guarded against.

Serum diagnosis is of no value and not resorted to although agglutinins are formed.

Immunity and Specific Therapy.—*Bact. tetani* produces an extracellular toxin of great potency. This toxin combines with the cells of the central nervous system. Certain animals as birds and the cold-blooded animals are naturally immune. This immunity is supposed to be due to the lack of receptors suitable for binding tetanus toxin. Whatever susceptibility man possesses undoubtedly depends on the suitability and affinity of receptors in the central nervous system for the toxin.

Vaccine Therapy and Active Immunization.—This is not used in man although by active immunization of horses tetanus antitoxin of great importance for man and the animals is obtained.

Serum Therapy and Passive Immunization.—In 1890 Behr-

ing and Kitasato immunized mice and rabbits to tetanus by injecting cultures of the bacillus of tetanus. These investigators found that blood from rabbits immunized to tetanus bacilli is able to protect mice against the disease. The first reliable antitetanic serum to be used in man was put on the market in 1896.

The process of producing antitetanic serum is similar to that employed in producing antidiphtheria serum—blood is drawn from horses that have received injections of increasing amounts of tetanus toxin. The toxin injected is produced by growing the tetanus bacillus on bouillon. After ten to fifteen days of incubation under anærobic conditions the bouillon culture is filtered through a Berkefeld filter. The germ-free filtrate contains the toxin which is present usually in large amounts. At the first injection into the horse usually 0.5 c. c. of toxic bouillon together with antitetanic serum are injected. The amount of toxin is increased at each injection until finally 700 to 800 c. c. of toxin are tolerated when given in one injection. After the third injection the antitetanic serum is usually omitted. After several months of treatment and complete recovery from the last injection, the horses are bled and the serum is collected. The serum is then concentrated and refined after the same methods that are used for diphtheria antitoxin.

The standardization of tetanus antitoxin until very recently has been indefinite and unsatisfactory. At the present time different standards exist in the various countries. The unit of antitoxin for tetanus for the United States has been fixed as that amount of tetanus antitoxin that will protect a 350-gram guinea-pig for 96 hours against one thousand times the smallest fatal dose of tetanus toxin. In order that the standard may be the same throughout the United States the Hygienic Laboratory of the United States Public Health Service sends out at regular intervals a stable precipitated tetanus toxin which is called the "test dose" and contains one hundred times the smallest fatal dose. It will be observed that a unit of tetanus antitoxin contains more than ten times as much neutralizing value as does a unit of diphtheria antitoxin. The various antitetanic serum producers furnish their product in

suitable syringes and as is the case with diphtheria antitoxin, state the date after which it is desirable that the serum should be returned if not used.

After the entrance of the tetanus bacillus usually there is little change produced in the tissues infected. Suppuration occurs practically only as a result of other organisms. Usually the organisms remain localized. The disease and its symptoms are entirely dependent on the absorption of the toxins produced by tetanus bacilli. Tetanus toxin circulates in the blood and lymph but shows no symptoms until it unites with and is absorbed by the end organs of the motor nerves and the central nervous system. To reach the end organs the toxin is carried through the axis cylinders. It is thus seen that when the symptoms of the disease appear the toxins have already combined with the cells, are exerting their toxic effects on them and can no longer combine with the antitoxin.

Tetanus antitoxin can only bind tetanus toxin before it is taken up by the nerve cells and axis cylinder. Because of this the value of tetanus antitoxin lies mainly in its prophylactic application. As a preventive of tetanus, tetanus antitoxin has proven to be of great value. Tetanus infection however is not always suspected until after the symptoms appear and for this reason tetanus antitoxin frequently can only be used for curative purposes. Treatment for prophylactic purposes differs to some extent from that for curative purposes.

For prophylactic purposes subcutaneous injections of antitoxin should be made when there are wounds and traumatism resulting from blank cartridges and fireworks explosions, nail punctures and injuries into which street, garden or barnyard dirt has been carried. The wound itself should be opened, cleaned and burned out preferably with fuming nitric acid. The dose of tetanus antitoxin in these cases is usually from 1,500 to 3,000 units given subcutaneously. It is advisable to make the injection as soon as possible because the incubation period may be short.

For curative purposes after the symptoms have appeared, much larger doses are given. Doses varying from 15,000 to 200,000 units have been injected. These injections are made

intravenously and even into the spinal canal and the subarachnoid space. Injections should be repeated until the symptoms abate. Meltzer, Kocher and others have used magnesium sulphate as an adjuvant in the treatment of tetanus. Meltzer uses a 25 per cent. solution, while Kocher advocates only a 10 per cent. solution. The solution is injected into the spinal canal and is of sufficient amount to equal 0.03 grams per kilogram of body weight. Kras¹, being unable to obtain antitetanic serum, withdrew 500 c. c. of blood and replaced it with salt solution and likewise removed the spinal fluid by puncture and replaced it by salt solution containing 0.3 per cent. of sugar. He repeated these procedures until recovery occurred.

While it is realized that tetanus antitoxin can only be of value as long as the toxin has not firmly combined with the receptors of the central nervous system, intensive antitoxin treatment is indicated as a curative measure. It has been claimed that by large doses of antitoxin applied directly to the toxin and cells to which it has become bound this union can be broken. Magnesium sulphate injections and removal of excess of toxin by bleeding and lumbar puncture must all be tried if the patient is to be given every benefit.

A dried tetanus antitoxin has been made. This powder is used principally as a dressing for wounds which are likely to be infected but it may also be used for injection after dissolving in salt solution. For the latter use it has the objection that it dissolves with difficulty. While relatively little is known concerning it, it has advantages similar to those of dried diphtheria antitoxin and may prove of considerable value to the medical profession.

Prophylaxis.—The prevention of tetanus concerns itself with the avoidance of infection through accident, in child birth, by sera and vaccines, and so on, and with early protective immunization. The value of immunizing doses of tetanus antitoxin cannot be definitely determined; if tetanus antitoxin is used successfully it is difficult to decide whether or not there has been infection with the tetanus bacillus. It is the belief of some however that tetanus can be prevented entirely if after

¹Kras: Wien. kl. Wchnschr., 1912, XXV, 88.

all injuries of the kind likely to be infected with tetanus bacilli, immunizing doses of tetanus antitoxin are injected early. Jordan states that in the United States in 1903 there were 4,449 Fourth of July injuries of which 406 cases died of tetanus while in 1907 when tetanus antitoxin was used quite universally for such wounds there were only 62 deaths caused by tetanus following 4,413 Fourth of July injuries.

As a result of the decreased number of cases of tetanus following Fourth of July wounds when immunizing doses of tetanus antitoxin are given the physician should in all cases of such injury administer to the patient an immunizing dose of tetanus antitoxin. Moreover all wounds in which tetanus infection might occur should be thoroughly cleansed and the patient immunized with tetanus antitoxin.

SMALLPOX.

Smallpox is an acute communicable disease occurring in epidemic and endemic forms. Before vaccination was introduced this disease caused the deaths of many persons and was regarded as most serious. The causal organism has not been discovered although many attempts have been made to do so. Guanieri, Councilman, Calkins and others have found protozoan forms in the lesions of the disease but their etiological importance is not generally accepted. Simpson¹ has isolated a diplobacillus and with this has inoculated and vaccinated calves. He considers this as an etiological factor in vaccinia.

Differential Specific Diagnosis.—Smallpox is a disease that occurs at all ages. It exists in different localities and when conditions are favorable increases to an epidemic form. When epidemics exist the diagnosis is easy but in the isolated cases the diagnosis is difficult at times because the severity varies a great deal, because it may be confused with chickenpox, and because in vaccinated persons it is likely to run only a mild course.

CLINICAL DIFFERENTIAL DIAGNOSIS.—The incubation period is from nine to fifteen days. The disease begins with chills, vom-

¹Simpson: Jour. Tropical Med. and Hygiene (London), 1912, XV, 209.

iting and fever. Fairly constant symptoms are frontal headache and lumbar pains. The temperature rises rapidly, there is vomiting, delirium and so on. After this skin lesions come on and the severe general symptoms subside to some extent. The skin lesions vary in the different stages. At first there is a scarlatinal or measly rash on the lower abdomen, inner sides of the thighs, and back and sides of the thorax. After three to four days this fades and the real smallpox eruption comes out. This begins usually first on the forehead and the hands and then involves the whole body. It starts as small, red spots which change to vesicles and on about the eighth to ninth day of the disease become pustular. On puncture the pustules do not collapse because of their cavernous structure. The pustules may remain discrete or become confluent. When these begin to recede the temperature again comes up to gradually recede by lysis after the twelfth day. When the eruption is confluent the fever remains higher and the general symptoms are more severe. At times there is a hæmorrhagic condition. If this comes on early the disease is severe and is usually followed by death.

The differential diagnosis may be difficult early in the disease because the rash resembles that of scarlet fever or measles. To differentiate smallpox from these diseases, angina in scarlet fever and coryza and Koplick spots in measles are of great value. The greatest difficulty comes in the differential diagnosis of smallpox and varioloid (smallpox in vaccinated persons) from varicella (chickenpox). Cases of varioloid should be quarantined as rigorously as smallpox. Chickenpox can usually be distinguished by the slighter illness, absence of shotty feel of the papules and presence of pocks in all stages of development. Smallpox may be mistaken for glanders. To differentiate these, inspection of the horses in the stable and bacteriological examinations are of the greatest value.

Bacteriological and serum diagnosis are of no value in smallpox. Jobling¹ has been able to get fixation of complement with blood from vaccinated calves and the vaccine virus used for inoculation. As soon as the causal organism of the

¹Jobling: J. Exp. M., 1906, VIII, 707.

disease is discovered methods of diagnosis other than clinical will undoubtedly be available.

Immunity and Specific Therapy.—One attack of the disease usually confers immunity although second and third attacks have been reported. It has been known for a long time that there is a difference in the virulence of different epidemics and that by the introduction of mild virus through the skin a mild form of the disease occurs which protects against further contraction of the disease. This was introduced into Europe by Lady Montague in 1718. From such intentionally inoculated persons however epidemics were started in certain cases.

In 1796 Jenner as a result of a good deal of study on smallpox and cowpox and from the observation that a person who has become inoculated with cowpox virus is protected against smallpox, inoculated a boy with virus from cowpox on a dairy-maid's hand. He found that in this way cowpox can be transmitted and immunity to smallpox be produced. Since then vaccination against smallpox by inoculation of cowpox virus has been quite universally adopted. It is now accepted that cowpox is a modified form of smallpox and that smallpox vaccination is a process of active immunization by means of living organisms attenuated by passage through cows.

Vaccination or Active Immunization.—Immunization according to the method of Jenner is known as vaccination and is done for prophylactic purposes.

The methods of obtaining virus for vaccination vary. Up to 1870 virus was usually obtained from the pustule of the vaccinated individual. Now however vaccine is usually obtained from young calves which have been inoculated on the abdomen and thighs with vaccine from the pustules of healthy children or calves. The pustules on inoculated calves are cleaned and opened on about the third or fourth day and from the material contained in them either so-called "vaccine points" or "glycerinated virus" is made. The animals from which the vaccine is obtained are kept clean and asepsis and antisepsis are employed as much as is possible. When all precautions are taken the vaccine still contains many organisms. Vaccine points are made by dipping sterile bone slips into the material from the

pustule. The vaccine on the slips is then dried and stored under aseptic conditions. Glycerinated virus is made by macerating the pustular material in glycerin and straining through gauze. Glycerin has the advantage of killing many of the bacteria and also makes it possible to put the virus into tubes. Before tubing, the virus is examined and tested in animals for *Bact. tetani*. Good vaccine contains few organisms and few species and after several weeks may contain no bacteria whatever.

Heat and age affect the potency and viability of vaccine virus. Some vaccines lose their potency in one month while others remain active for three to four months. The labels on vaccines state the date after which the vaccine should not be used.

In 1910 Calmette and Guérin studied the effects of vaccine virus on rabbits. Pfeiffer and Voigt from this work developed what they call "lapine" which is a vaccine obtained from rabbits inoculated with cowpox virus. It has no especial value over cowpox virus except that it makes it possible to get vaccine in such countries where calves are not available.

The method of vaccination varies. It is the custom now to produce scarifications of one-eighth to one-fourth of an inch in diameter by repeated light scratches with a needle on the well-cleaned arm or thigh. When vaccination is made on the arm the area selected is about the point of insertion of the deltoid muscle. In cleaning the part where scarification is to be made it is to be remembered that all antiseptics must be removed with sterile water before inoculation is made. Scarification is only made severe enough to produce an exudation of serum, bleeding being avoided as much as possible. After scarification vaccine on the point or from the tube is well rubbed in, the serum is allowed to dry and a dry sterile dressing is put on.

The reaction produced by successful vaccination usually appears after about three to five days. Locally there is at first a papule which becomes a pustule on the eighth or tenth day. About the end of the second week the vesicle changes to a scab which comes off and leaves a scar. Constitutional symptoms usually appear about the third day and last until the end of the

first week after vaccination. When there is infection cellulitis and sloughing follow; these conditions are treated as any other infection but usually ought not occur.

Von Pirquet has called attention to the differences between reactions of the first and subsequent vaccinations. While the first vaccination on the first day shows slight trauma which then disappears until the real reaction appears on the third to fifth days, on revaccination a reaction appears within a very short (24 hours) time or not at all. When a revaccination "takes" there is less fever and the duration of the reaction is shorter. These observations were made by Jenner but were not recognized in the light of allergy as they have been by von Pirquet.

The duration of immunity varies and is uncertain. The longest time that immunity can be certainly relied upon is two years. The rule for vaccination which is generally adopted and advised consists in vaccination within the first or second year and certainly before entering school, revaccination within the tenth to fifteenth year and after that whenever there is an epidemic of smallpox or possibility of exposure to the disease.

The efficiency of vaccination against smallpox cannot be doubted. The mortality in vaccinated individuals is between 5 and 8 per cent. while in the unvaccinated 35 to 40 per cent. of the cases terminate fatally. The disease thus is milder in vaccinated than in unvaccinated individuals. Moreover fewer cases of smallpox occur in the vaccinated than in those not vaccinated.

Serum Therapy and Passive Immunization.—That blood sera of man and animals that have been immunized to smallpox contain antibodies is no longer doubted. Transference of immune bodies in sufficient amounts to be of value for curative purposes has not been successful.

Prophylaxis.—Vaccination for protective purposes has already been described. In the minds of most people there is little doubt of its value. Objections to the methods have however been strenuous among some people. These are based either on undesirable by-effects of vaccination or the result of certain isms. Against the first we can guard by using

only standard vaccines for immunization, never carrying the virus from one person to another and using strict asepsis during vaccination and at the time of reaction. To combat the isms our problem is more difficult. The health authorities should assert their right to compel vaccination if children are to be permitted to go to school. When this cannot be done it will devolve on the laity to ostracize unvaccinated persons especially at the time of occurrence of the disease in a community.

RABIES, HYDROPHOBIA OR LYSSA.

Hydrophobia is a disease the etiology of which is only indefinitely established. The organism responsible for the condition has never been grown. By some investigators certain peculiar bodies found in 1903 in the large nerve cells in the central nervous system are regarded as the causal factors in the disease. Although these bodies are not universally accepted as the etiological factors they are quite generally accepted as being specific to this disease.

The communicability of hydrophobia was proven by Zinke in 1804. The disease occurs especially in dogs and man but also may be communicated to cattle, cats, horses, pigs, rabbits and so on. The general symptoms are the same in man and animals but there is a marked difference in the incubation period. This difference is not only dependent on species however for in the same species symptoms come on sooner when infection occurs about the face and head.

Differential Specific Diagnosis.—Hydrophobia is communicated to man largely by dogs. Treatment of the disease once it has developed is of slight avail but immunization after contraction of the disease and before the appearance of symptoms is most efficient. For these reasons the diagnosis of the disease in animals is of more real importance than is the diagnosis in man.

CLINICAL DIFFERENTIAL DIAGNOSIS.—**THE DISEASE IN DOGS.**—It has already been mentioned that the incubation period varies in different animals. In dogs the incubation period is from three

to six weeks but may be from five to seven months. Raving and quiet types of the disease are recognized. As to which the animal will have depends somewhat on the temper and training of the animal, the quiet form occurring most frequently in well-dispositioned dogs. In the beginning of the symptoms the animals are restless, may be very friendly but usually are tricky and snap when not watched. The appetite is poor although indigestible things are frequently eaten. After one to three days the animal begins to rave and bite, howls with a hoarse voice and has spasms of the muscles of the larynx so that the drinking of water is impossible. At this time there is salivation leading to the frothing at the mouth. After three to four days of this the animal becomes paralyzed usually first in the hindquarters and four to six days later dies. In the quiet form the animal does not run about, paralysis may set in earlier and the danger of biting man or animals is slight.

THE DISEASE IN MAN.—In man the incubation period varies according to the location and severity of the bite and amount of virus introduced. It is from 15 to 60 days but may be longer. The regular symptoms are preceded by prodromal symptoms in which there is pain and irritation about the bite, the patient is depressed, has headache and loss of appetite. At this time patients are likely to be irritable and sensitive to things otherwise not observed. In the disease itself there usually first comes difficulty in swallowing and later spasm of the respiratory muscles. The patient becomes more fretful, may be delirious or maniacal. Salivation occurs and there is a marked desire for water. After several days paralysis comes on and the patient dies by syncope.

While it usually is easy to diagnose rabies in animals and man we cannot wait to make a clinical diagnosis in man to make specific treatment of value. For this reason animal inoculations and search for Negri bodies must be made.

DIAGNOSIS FROM ANIMAL INOCULATIONS.—This is the safest and most reliable method for the diagnosis of the disease. Man usually is infected from animals especially the dog. For animal inoculation the spinal cord or medulla of the suspected animal is taken out under aseptic precautions, macerated in salt

solution and a part of the emulsion is injected into the subdural space through a trephined hole in the skull of a rabbit. If there has been degeneration of the cord subcutaneous injections should be made. The result in rabbits is absolutely dependable. In positive cases the incubation period in the rabbit is usually from 12 to 15 days but may be longer. When the disease develops the animal runs against the sides of the cage, gradually becomes paralyzed and dies. Injection of cord from this rabbit will produce the disease in other animals.

DIAGNOSIS FROM NEGRI BODIES.—In 1903 Negri reported that he had found in the central nervous system, especially Ammon's horn, of persons and animals dying of lyssa certain intracellular bodies. These have since been known as Negri bodies. They are round, oval or elliptical cells and usually vacuolated. For purposes of diagnosis the skin of the head of the dog should be removed, the operator being careful not to infect himself through old wounds or by injury during the operation. After the skin is removed the outer tables of the skull are sawed in a line running from the foramen magnum to the frontal sinus on each side. After this the bones can be completely removed with the chisel and mallet and the brain taken out. Bits of tissue are taken from the hippocampus or Ammon's horn and the plexiform ganglion. With these tissues smears are made on a number of slides and some of the tissues are hardened and fixed for cut sections. For making the smears pressure with another slide may be necessary.

Many different methods have been devised for staining for the bodies. A most satisfactory method is to fix in Zenker's fluid for one-half to two hours, wash in water, immerse in 95 per cent. alcohol for 5 to 10 minutes, then in saturated alcoholic solution of iodine for 5 to 10 minutes, wash out the iodine with 95 per cent. alcohol, then wash in water, stain for 5 minutes in 5 to 10 per cent. watery solution of eosin (Grübler, W. G.), wash in water, stain 2 to 3 minutes in Unna's polychrome methylene blue, wash in water, differentiate in 95 per cent. alcohol, blot, dry and examine under the oil immersion. The Negri bodies with this stain take a magenta color, have a vacuolated appearance and dark blue granules.

Serum Diagnosis is not of much value. Complement fixation has been obtained with the serum of immunized persons but the results are not reliable.

Immunity and Specific Therapy.—Natural immunity is possessed by some animals as the reptiles. In man and the warm-blooded animals there is little immunity. It of course happens that only a few of the animals or persons bitten by a mad dog will contract the disease but this may be associated with failure to really introduce the virus into the tissues. The duration of immunity acquired by the Pasteur treatment has not been determined satisfactorily.

Active Immunization and "Pasteur Treatment."—The inability to establish the etiological factor in the disease has not prevented the establishment of methods of immunization. The principle of the method of immunization most generally practised is based on the establishment of an active immunity during the period of incubation of the disease. In man the period of incubation usually lasts from four to six weeks though it has been found to be as short as fifteen days and as long as one year. The relatively long period of incubation makes it possible to establish an active immunity before the symptoms of the disease develop.

Immunization is accomplished by repeated injections of attenuated "fixed virus." Pasteur found that tissues and fluids from rabid animals vary considerably in virulence and that the virulence of virus can be changed. If successive reinoculations into rabbits are made from the virus of a rabid dog the virus is increased in virulence for rabbits. After a number of passages through rabbits the virus finally can no longer be exalted in virulence. This is then called the fixed virus, is usually obtained from the cord and produces symptoms in a rabbit in six to seven days. This fixed virus can by drying be gradually decreased in virulence so that after fourteen days of drying it has lost all virulence for rabbits. To immunize the individual virus of low virulence is first injected, after which injections are made with virus of greater virulence until at the end of the treatment injections of cord which has dried only from two to three days are made.

At the present time there are in the United States many so-called "Pasteur Departments" for immunization against rabies. The particular technique employed in such departments varies in some details still the fundamental and essential principles are the same.

To prepare virus according to the original Pasteur method rabbits are inoculated intradurally with the medulla of fixed virus. After six to eight days the animals develop symptoms and die four to five days later. The dead animals are sprayed with lysol, the hair removed and under aseptic precautions the brain and cord are taken out. The cord is separated from the medulla, divided into two parts and with sterile silk gauze is suspended in a glass jar at the bottom of which is flaked caustic potash. The medulla is used to inoculate new rabbits. The dead rabbit is examined to make certain there has been no other disease. The cord suspended over potassium hydrate is now stored for the required length of time. For injections the required amount of cord of the right age is cut off and mashed with a glass rod, then water is added until a milky fluid results. The sediment soon falls out. Harris has dessicated the virus at very low temperature and in this way preserves virulence for long periods of time. Pasteur's original method was to inject first cord that had dried 15 days and on each successive day to inject less attenuated virus until finally cord that had dried only 5 days was injected. At present cord that has dried more than 13 days is seldom used. Babes has even injected cords dried only one day and Marie has advocated the simultaneous injection of fixed virus and the serum of sheep immunized to lyssa. It is difficult to judge on the value of the different schemes followed because undoubtedly many people receive the treatment without having the infection. Generally however the treatment is for 15 to 20 days and should be instituted as early as possible. While formerly patients had to go to the different hospitals providing Pasteur treatment, rabies vaccine is now sent out from laboratories. The injections are made into the cutaneous tissue and under strict aseptic precautions.

Serum Therapy and Passive Immunization.—The serum of immunized animals contains substances that render the virus

inert. The method of Marie has already been mentioned. Immunity apparently is more quickly induced by his scheme of simultaneous injection of virus and cord. Immunization against rabies has been attempted by the injection of antirabic serum. Babes and Lepp in 1889 found that they could protect dogs against rabies by injection of serum from dogs actively immunized to rabies. Tizzoni and Centanni have reported favorable results with antirabic serum. The value of these newer methods has not been proven so that at the present time conclusions as to their value are impossible.

Prophylaxis.—Prevention of hydrophobia is our most potent remedy. The Pasteur treatment is undoubtedly of the greatest value but to get the best results early diagnosis of the disease in animals especially dogs is essential. To accomplish this the dog should be corralled and kept under observation. If symptoms of rabies appear he should be killed in such a manner as will not injure the head. From the proper parts of his brain smears should be examined for Negri bodies and rabbits or guinea-pigs inoculated. If the dog's head is to be sent to a laboratory it should be packed in ice. As soon as a tentative diagnosis of rabies is made all persons bitten should be given the Pasteur treatment. There are no persons with specific contraindications. There are some ill effects from the treatment but these are usually of a transitory nature and may be due to the virus in the bite and not that given in the treatment. The symptoms come on at the end of the first week of treatment and are insomnia, headache, numbness in the legs and pain at the sites of injections. The paralysis may become grave and involve a large part of the body but usually clears up rapidly and entirely. Abscesses and local infections are seldom produced by the injections. All of the symptoms present may be due not to the treatment but to the infecting virus and even if they are due to the treatment no one who has ever seen a patient in the severe stages of hydrophobia will hesitate to decide to submit himself or his patients to the treatment rather than to the disease.

Because of the frequency with which dog bites are by animals having hydrophobia, the wounds should at once be cauterized

with fuming nitric acid and the fluid drawn out by Bier's method.

HAY FEVER, ROSE FEVER, AUTUMNAL CATARRH.

This is a disease occurring only in certain persons and is an infection of the upper air passages often accompanied by asthmatic attacks. For a long time the nature of the disease was not understood. Bostock believed it to be due to summer heat. Elliotson suspected pollen of certain plants but it was not until Dunbar and his associates Luebbert, Prausnitz, Kamman and others demonstrated that the pollen of certain plants when brought into contact with the mucous membranes of persons susceptible to hay fever produces irritations identical with those in natural hay fever. According to Dunbar the reaction is due to a toxalbumin separated from the pollens by precipitation with salt and water. This however did not solve the problem entirely for the toxalbumin does not affect well persons and it was not until allergy was understood that the causal factors in the disease were really known. The disease then according to our present view is due to allergy (formerly regarded as idiosyncrasy) and the special toxin of certain plants. The especial common plants bearing toxic pollen are: ragweed, golden rod, honeysuckle, lily of the valley, chrysanthemum, aster, evening primrose, rye, oats, barley, rice, wheat and most of the grasses.

Undoubtedly not all of the diseases included under the term are due to pollens for the condition of the nasal mucous membrane, nasal polypi and so on play a part in some cases.

Differential Specific Diagnosis.—CLINICAL.—The disease occurs especially in the spring and fall. It affects only certain individuals and exists in certain families. Clinically it is not unlike coryza but there usually is more headache, cough, sneezing and asthma.

ALLERGY OR HYPERSENSIBILITY TEST.—Dunbar has prepared the taxalbumin for diagnostic purposes. It is provided in the dry form consisting of 0.0002 grams of toxin and 0.018 grams of sodium chloride. From this various dilutions are made and instilled into the eye. At first 1 to 100,000 dilution is put in. If no reaction

appears in 10 minutes a dilution of 1 to 50,000 is tried. If still no reaction occurs dilutions as low as 1 to 1,000 may be tested with. A positive reaction is manifested by congestion of the blood vessels and suffusion of the caruncle. The reaction may be stopped by putting in a drop of the antiserum.

Immunity and Specific Therapy.—Sensitization is necessary to have the affection. When this occurs is not determined but in many cases it is inherited. In many cases after the disease has existed for a time it disappears.

Specific treatment is along several lines: the neurotic condition must be overcome, attempts must be made to remove local irritation and Dunbar's antitoxic serum (Pollantin) should be used.

Pollantin is obtained from horses immunized to the toxin. It is standardized and is dispensed in the liquid and dried forms.

Pollantin is applied locally to the eye, nose and throat. The effects if the serum is of value show immediately but wear off after a short time. It is not successful in all cases. Patients in whom the treatment is successful should carry the serum with them. Serum anaphylaxis has resulted from the use of pollantin and in such cases very dilute solutions should be used.

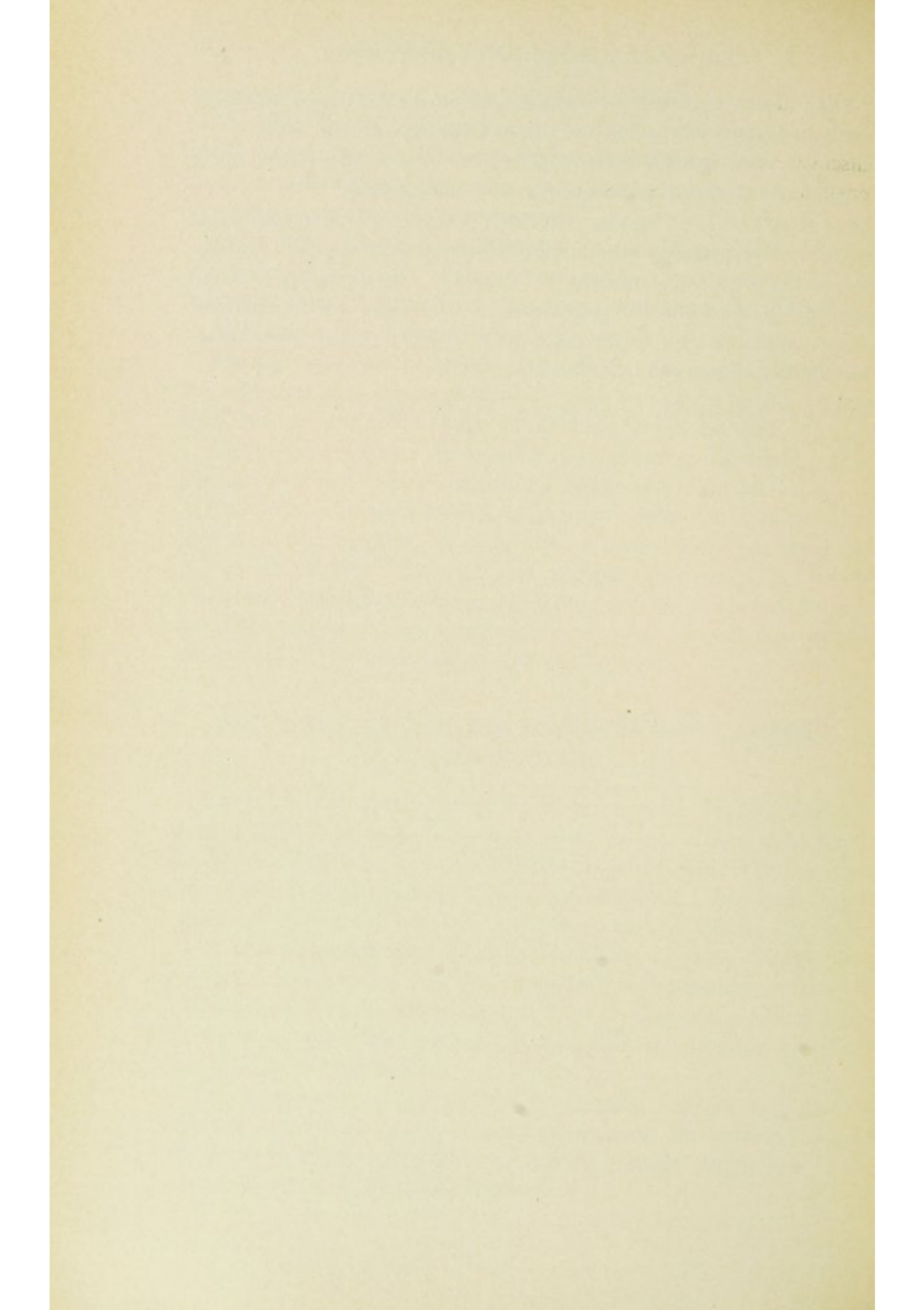
BACTERIA OF LESSER IMPORTANCE CAUSING INFECTION.

There are a number of other infections, as with *Bac. pyocyaneus*, pseudodiphtheria bacilli and so on, that have not been considered in detail. The organisms are not the causes of clinical entities but frequently take part in local and mixed infections. For their diagnosis bacteriological methods are used. Serum therapy in these is of little value although vaccines have been of value in many instances. Dentists use vaccines extensively in pyorrhœa, aurists in infections of the ear, and so on. Whenever they are used it is best to use also such other methods as are generally accepted as being of value in the treatment.

For a number of animal diseases specific vaccine and serum therapy have proven of great economic value as for anthrax, swine plague, hog cholera, symptomatic anthrax, cattle plague and to a certain extent in tuberculosis of cattle.

The causal organisms of measles and chickenpox have not been discovered at this time but our knowledge concerning them is increasing. The investigations of Flexner, his assistants and others on epidemic anterior poliomyelitis will undoubtedly help to solve the problems of this disease. Recently Flexner and Noguchi¹ have made in their fifteenth note a short communication on the cultivation of the organisms causing the disease. This probably will not only lead to methods for cultivation of the organisms of epidemic poliomyelitis but also to the cultivation of some of the other ultra-microscopic organisms and filterable viruses.

¹Flexner and Noguchi: J. Am. M. Ass., 1913, LX, 362.



APPENDIX.

INFECTIONS WITH SPIROCHÆTA (Treponema) PAL- LIDA (Causal Organism of Syphilis).

It is needless and almost impossible here to consider all of the organisms that have been held responsible for syphilis. The disease has long been known to be communicable but it was not until Metchnikoff and Roux showed that apes are subject to inoculation with syphilitic virus that the susceptibility of certain animals was proven. Of all of the organisms Lustgarten's bacillus, resembling the tubercle bacillus, occupied the most prominent position for some time as the possible cause of the disease. In 1905 Siegel reported in various places that he had found in the blood and organs of syphilitics certain protozoan forms which he called cytorrhynes. Schaudinn and Hoffmann², in trying to confirm Siegel's observations discovered *Spirochæta pallida* which has since been regarded as the causal organism of syphilis.

Differential Specific Diagnosis.—Syphilis is a disease usually of slow development. Two modes of infection are recognized and spoken of as acquired syphilis and congenital syphilis. Acquired syphilis is usually the result of infection during coitus but may also be contracted extragenitally as a result of kissing, wet nursing, in surgical and confinement practice, during autopsy and so on. Formerly when smallpox vaccination was made by transference of virus from one patient to another the disease was at times distributed in this way. Congenital syphilis is acquired in utero when the father or mother or both have syphilis. The closer in time the conception is to the occurrence of the primary infection the greater the possibility of the foetal infection. A mother free from syphilis on bearing a syphilitic child becomes immune. In the acquired form of the disease several stages are distinguished and usually occur: the primary lesion at the point of infection; the

¹Metchnikoff and Roux: Ann. de l'Inst. Pasteur, 1903, XVII, 809.

²Schaudinn and Hoffmann: Deutsche med. Wchnschr., 1905, XXXI, 711, 1665.

secondary lesions manifested by constitutional symptoms as affections of the skin and mucous membrane; the tertiary lesions characterized by granulomatous growths in the bones, skin, muscles and viscera; and the parasyphilitic lesions manifesting themselves especially as diseases of the nervous system. The different stages and lesions do not occur in all cases of the disease even when the disease is untreated.

CLINICAL.—Syphilis must be diagnosed in the various stages. As far as the clinician is concerned it must be remembered that syphilis may attack practically any part or tissues of the body and the symptoms and signs produced vary a great deal. The primary lesion appears from ten days to three months after the infection but in most cases appears in three weeks. The genital chancre is usually *single*. It generally appears as a superficial erosion, becomes indurated, has a smooth dusky-red surface, a slight serous exudate and even without treatment goes on toward cure. In the inguinal region the glands are enlarged but usually painless. The extragenital chancres also are usually single, are chronic and refuse to heal. Infections of the finger obtained during autopsy or at operation are extremely painful and although the lesion may be small there is much induration of the finger pulp. Chancre of the lip may involve the whole lip and is followed by enlargement of the submaxillary glands. On the tongue the ulcer is erosive and covered with a pseudomembrane. In the secondary stages there is anemia (reduction of numbers of red blood cells and of hæmoglobin content), general glandular enlargement (enlargement of the epitrochlear glands being regarded as an especially diagnostic point), moderate fever up to 101° F., muscular and articular pains, alopecia causing regular and irregular bald spots, eruption of the mucous membrane (mucous patches) and of the skin. Of the skin eruptions the special features are that they come on slowly, there is no itching, they are symmetrically distributed, superficial and usually copper colored. The secondaries come on about forty days after the primary sore. In the tertiary stage there is ulceration and involvement of the deeper tissues, gummata may be found in the skin, subcutaneous tissue, muscles and internal organs. In the internal organs amyloid degeneration frequently occurs. It is hard usually to determine when the secondary stage ends and the tertiary

begins. The parasyphilitic manifestations are locomotor ataxia, dementia paralytica, epilepsy, cerebral hæmorrhage, aneurism and so on. These conditions it must be remembered also occur without syphilis.

Congenital syphilis is not always manifest at birth but may appear after a short time. The distinguishing characteristics at birth are a small baby weighing only or less than four times as much as the placenta instead of six times as much. The placenta itself may be of a dull greasy appearance and the villi are clubbed and thick. The child may have lesions about the wrists, ankles, hands and feet. "Snuffles" or syphilitic rhinitis is common and there is depression at the root of the nose. The spleen and liver are enlarged. If the child lives it usually does not do well. At the time of second dentition the upper central incisors are peg-shaped and narrowed at the cutting edge (Hutchison's teeth). Later in life iritis, keratitis and bone diseases especially of the periosteum of the tibiæ develop. For more details in regard to the symptoms and signs the reader is referred to the text-books.

MICROSCOPICAL AND CULTURAL DIAGNOSIS.—With the definite establishment of *Spirochæta pallida* as the etiological factor in syphilis the demonstration of the organism has become the most reliable means for the diagnosis of syphilitic infection. Until methods for the cultivation of the organism were devised stained preparations, observations in the hanging drop and better under the dark-field illuminator had to be relied upon for the identification of *Spirochæta pallida*.

Spirochæta pallida are not easily found in all syphilitic lesions, therefore the choice of material for examination is important. The organism can practically always be demonstrated in the secretion of the primary sore. To obtain the secretion, the sore and surrounding tissues should be well washed with sterile salt solution and then with a platinum needle or some other needle or knife slight scarification of the sore be made until the serum exudes. With Bier's suction cups serum may also be obtained. The serum is spread on slides and properly stained. If calomel, iodine or other medicinal applications have been used, these should be washed off and several days after local treatment has been discontinued the serum should be obtained. The organisms are also quite easily

demonstrated in the fluid obtained by hypodermic puncture of the enlarged glands. For the demonstration of organisms in the circulating blood Noeggerath and Staehelin¹ recommend that at least 1 c. c. of blood be mixed with ten times as much one-tenth per cent. acetic acid solution and then centrifugated at great speed. The sediment will contain *Spirochæta pallida*.

In the secondary lesions such as condylomata, skin eruptions and mucous patches the organisms can generally be found. It is necessary usually to clean the surface and obtain fluid but in doing this it is advisable to avoid bleeding. In the lesions of the deeper organs the pallida are less easily found. In congenital syphilis the organisms are found usually in great numbers in the liver, lungs, heart muscles and other organs. The placenta usually also contains the *Spirochæta pallida*.

The smears for stained preparations must be very thin. Fixation is usually accomplished by 15 to 20 minutes of exposure to absolute alcohol, although formaldehyde and other chemicals or slight heating have been used in some laboratories. Various stains have been recommended. The best known is the Giemsi stain which consists of azure ii 0.8 gram, azure ii eosin 3.0 grams, glycerin 250 grams and methyl alcohol 250 grams. Two methods of staining are used. The slow method requires four to twelve hours; the rapid requires dilution of the stain with distilled water and several exposures of 15 to 30 seconds to the heated stain. Other methods are to stain with hot gentian violet, to expose to 10 per cent. silver nitrate solution for 10 to 12 hours in the light, and so on.

The diagnosis of *Spirochæta pallida* from stained specimens is not always easy because of other organisms that closely resemble them. The organism stains with difficulty, is a spiral structure 6 to 15 micra long, is very slender, the windings are close and steep, and at both ends the windings are not as high as in the middle thus giving the organism a tapering appearance. *Spirochæta refringens* stains more deeply and has coarser and flatter windings. Noguchi² has isolated two treponema from the oral cavity, of which *Treponema microdentium* may be confused with *Treponema pallidum*.

Identification of *Spirochæta pallida* is somewhat easier in the

¹Noeggerath and Staehelin: München, med. Wehnschr., 1905, LIII, 1481.

²Noguchi: J. Exper. M., 1912, XV, 81.

hanging drop under the dark-field and examined with the oil immersion lens. The organisms are motile, rotate about the long axis, move forward and back and become angular but do not change their actual position very much. They refract the light only slightly.

The best positive diagnosis of syphilis is however based on the cultivation of the organisms. This has been accomplished by Schereschewsky, Mühlens and Hoffmann, Noguchi¹ and others. To get growth of the organisms Noguchi finds two conditions are all important, strict anærobiosis and recognition of the fact that the pallida migrate in the solid medium in which they are multiplying. To meet these conditions he uses tall test tubes into which he first puts a fragment of sterile rabbit kidney or testicle and then adds a considerable amount of 2 per cent. slightly alkaline agar and one part of ascitic or hydrocele fluid. This he inoculates with material obtained from lesions rich in spirochætæ. Only small pieces are used preferably of the chancre, condyloma or skin papule. The tissues are washed with sterile salt solution and immersed in sterile salt solution containing 1 per cent. sodium citrate before the medium is inoculated. For inoculation he used a glass rod or long heavy platinum loop and with this he pushes the tissue far into the medium. After inoculation the medium is covered with about a half-inch layer of sterile paraffin oil. The tubes are incubated at 37° C. for two to three weeks. The first cultures are usually impure. With a fine capillary pipette material is taken from the different parts of the medium away from the line of inoculation and examined under the dark-field. If spirochætæ are present a number of new tubes are inoculated deeply into the medium by means of the capillary pipette. After some time of incubation a number of the tubes will show a hazy growth away from the line of the stab. This is usually produced by the *Spirochæta pallida*. From this another transfer is made and usually a pure culture obtained. The pure cultures have distinctive properties; the colonies are plainly visible, seldom discrete, the growth is most marked about the fragment of sterile tissue and there is no odor produced. Noguchi² and Hoffmann³ have proven the pathogenesis for the rabbit. Inoculations are also successful in monkeys.

¹Noguchi: J. Exper. M., 1911, XIV, 99; and 1912, XV, 90.

²Noguchi: J. Am. M. Ass., 1911, LVII, 102.

³Hoffmann: Deut. med. Wchnschr., 1911, XXXVII, 1546.

SERUM DIAGNOSIS.—Serum diagnosis of lues is of the greatest interest to the practitioner at the present time. Since the discovery of *Spirochæta pallida* many attempts have been made to devise tests for the serum diagnosis of the disease. Specific agglutination and lysin production in the disease has been claimed. Of greatest promise and interest has however been the application of the fixation of complement reaction of Bordet and Gengou as made by Wassermann, Neisser and Bruck, Detre and others. The fixation of complement reaction, its advantages and shortcomings, the great care and control tests necessary and so on have already been considered on pages 59 to 65. The reader is referred to these pages for the details of the test.

The so-called Wassermann test however is not a definite fixation of complement reaction in the bacteriological sense for the antigen used need not be of the spirochaetæ themselves, not even from lipotropic substances found in the tissues of syphilitics but may be obtained by extraction from some of the tissues of normal individuals. The Wassermann reaction then in the first place is not an antibody-antigen reaction as is the complement fixation reaction of Bordet and Gengou. This does not invalidate the test however especially so since Noguchi¹ has found that there is fixation of complement with antigen prepared from his cultures of *Spirochæta pallida* and the blood serum of patients with latent and tertiary syphilis.

The original Wassermann test was made by using antigen prepared from fresh organs of syphilitics. The extraction was made by shaking for 24 hours in 0.5 per cent. carbolic acid in normal salt solution and then centrifugalizing to throw down the cells and bacteria. The antigens now used are chiefly alcoholic extracts. Noguchi and Bronfenbrenner² have studied these extracts and find that the alcoholic extracts of the liver tissues of man and certain animals on fractionation may be divided into four groups: (1) substances insoluble in ether and hot alcohol and not antigenic for the Wassermann test; (2) substances insoluble in ether and soluble in hot alcohol and with slight antigenic properties; (3) substances soluble in ether, alcohol and acetone, this fraction is antigenic but also hæmolytic and anticomplementary; (4) substances insoluble in

¹Noguchi: J. Am. M. Ass., 1912, LVIII, 1163.

²Noguchi and Bronfenbrenner: J. Exper. M., 1911, XIII, 43.

acetone, antigenic and usually non-hæmolytic but may be anticomplementary.

Noguchi uses as antigen in syphilis diagnosis the acetone insoluble fraction of an ether or alcoholic extract of normal liver or heart muscle. Keidel and Hurwitz believe extracts of syphilitic tissues are superior to extracts of tissues from healthy individuals. It must be evident that though there is controversy as to whether antigen should be obtained from the tissues of healthy or syphilitic organs, the value of a fixation of complement test in syphilis depends to some extent upon the method of extraction and the fraction used in the test.

In the original Wassermann test the hæmolytic system consists of sheep corpuscles and antishæp amboceptor. Noguchi found that human serum contains a variable amount of natural amboceptor for sheep corpuscles capable of being reactivated by guinea-pig complement and that because of this syphilis antibody may be missed. To overcome this he advocated the use of a hæmolytic system consisting of human red corpuscles and antihuman amboceptor. This is now used in most laboratories.

The technique for doing the test varies in the different laboratories but conforms generally to the following: From 2 to 10 c. c. of blood are taken under such precautions as will assure sterility, for certain bacteria are hæmolytic. The serum is allowed to separate out, is drawn off, diluted with salt solution and inactivated by heating to 56° C. for one-half to one hour. Fresh guinea-pig blood is obtained by hypodermic puncture into the heart of the anæsthetized living animal. The blood is allowed to clot, is drawn off and diluted 10 times with normal salt solution. The parts of the hæmolytic system are prepared as has already been described (page 63). The strength of antigen, complement and amboceptor must be determined. Noguchi has prepared and standardized filter paper strips saturated with each of these solutions. In this form they do not deteriorate much and may be used for some time after preparation. For measuring the quantities of fluids used in the tests graduated pipettes of fine calibre should be used. The mixtures should be shaken from time to time while the test is in progress.

The following table shows a laboratory record of a test without the control tests which have been mentioned before :

Antigen solution	Patient's serum	Complement (dil. 1:10)	Salt solution		Antihuman serum (dil. 1:100)	Human corpuscles in suspension (10 per cent)	Hæmolysis	Fixation of complement (Diagnosis)
0.5	0.2	0.5	0.3	Incubate one hour at 37° C.	1.0	1.0	None	++
0.5	0.15	0.5	0.35		1.0	1.0	None	++
0.5	0.10	0.5	0.4		1.0	1.0	50 per cent	+
0.5	0.5 (1:10)	0.5	0.0		1.0	1.0	20 per cent	+ —
0.5	0.1 (1:10)	0.5	0.4		1.0	1.0	Complete	—

The reading of the reaction is easy when properly controlled but it must be evident that in using the reading as a means of diagnosis of syphilis there are two important difficulties. Of these the first concerns itself with what shall be called a positive reaction for there are different degrees of hæmolysis and different amounts of the same serum give different reactions. The second difficulty met with is to decide whether the different degrees of hæmolysis and the different amounts of serum necessary to produce a positive reaction are of the same importance in all cases of syphilitic infection. Many attempts have been made to solve these questions but in the minds of the real investigators on this subject the interpretation of the reaction varies in the different cases. Three essentials are necessary to decide that the patient has or has not syphilis; the test must be done by one skilled in the technique and having a knowledge of all factors playing a part in the reaction, the test must usually be repeated after an interval and all the facts concerning the patient's history, treatment and habits must be known. Without knowing

all of these things a diagnosis of syphilis as based on the fixation of complement test should not be ventured.

The occurrence of positive fixation of complement reactions in the various stages of syphilis have been reported by many investigators. Some of the reports are of little value because the various factors mentioned in the preceding paragraph have not been considered or mentioned. Recently some valuable data have been reported by Craig.¹ His results are based on the study of 10,000 tests made in the Army Laboratory in Washington and are shown in the following table:

RESULT IN WASSERMANN TEST IN 3,381 CASES DIAGNOSED AS SYPHILIS OR HAVING SYMPTOMS SUGGESTIVE OF THE DISEASE.

Stage of the Disease	Number of Cases	Number Positive	Number Negative	Per cent Positive
Primary	654	580	74	89.4
Secondary	1434	1372	62	95.4
Tertiary	465	404	61	86.8
Latent	778	509	269	65.4
Congenital ...	28	25	3	89.2
Parasyphilitic.	22	15	7	68.1

From the table it will be seen that in latent syphilis the percentage of positive reactions is lowest and in the secondary stage it is highest.

The appearance of the reaction in the primary stage increases gradually to the fourth and fifth weeks. During the first week only about one in four patients give the reaction. During the primary stage a reaction showing considerable inhibition of hæmolysis is suggestive if there is a history of infection and there is a suspicious sore but the test should be repeated later. In the secondary stage there usually is a good reaction so that a positive diagnosis should not be made if there is only partial hæmolysis. In the tertiary stage and in latent syphilis a negative reaction is of little diagnostic value unless it remains so over a period of several months, while

¹Craig: J. Am. M. Ass., 1913, LX, 565.

partial hæmolysis only warrants further examination. In congenital syphilis the reaction is usually clear cut and positive. The same holds true of paresis while in tabes only about one-half the patients give a positive fixation of complement reaction. In the tabes cases the test should be tried with the cerebrospinal fluid but it must be remembered that four to six times as much spinal fluid must be used as of blood serum.

The influence of specific antiluetic treatment on the reaction has been much discussed. Earlier some of the substances interfering with the complement fixation test have been mentioned (page 65). These and antiluetic treatment certainly lead to the disappearance of a positive reaction. The test has been utilized to control the treatment and is generally regarded as an index of the efficiency of the treatment. If the reaction remains positive, or even partially so, further treatment should be given. One negative reaction after treatment however is of no value as far as evidence of cure is concerned. Before cure can be assured there must be no symptoms and the reaction must have been negative at least one year after the cessation of treatment. Craig and Nichols have shown that drinking of alcohol as in whisky and so on causes the disappearance of the reaction for several days. This should not be lost sight of when a diagnosis of syphilis is based on the Wassermann reaction.

Positive Wassermann reactions have been found to occur in patients not having syphilis. The principal other diseases in which a positive reaction has been obtained are scarlet fever, leprosy, sleeping sickness, lupus, malaria, carcinoma and yaws. In many of the tests showing a positive Wassermann reaction the amount of patient's serum used has been larger than is ordinarily used in a test for the diagnosis of syphilis.

The *real value* to be attached to the result of the Wassermann test in the diagnosis of syphilis depends on proper performance of the test, proper interpretation of the reaction and knowledge of the patient's history, symptoms, signs, habits and treatment he has received. When this is realized we cannot help but feel that much harm as well as good has come from the test. All over the country are laboratories advertising that Wassermann tests are made. Physicians send blood specimens to these laboratories and receive a re-

port reading "syphilis absent" or "syphilis present." Fortunately while the reaction is not specific in the bacteriological sense properly performed it is clinically characteristic of syphilis and therefore the diagnosis made is frequently correct. Diagnosis based on fixation or non-fixation of complement alone is however not always right and undoubtedly persons with syphilis but diagnosed as being free from syphilis have caused further infection and persons free from syphilis but diagnosed as having the disease have been compelled to expend considerable sums of money for treatment, suffer the mental depression that comes when this dreaded disease is diagnosed and in many cases such diagnoses have led to separation of husband and wife and estrangement of parents and children. The test should preferably be made in well-equipped laboratories. The interpretation of the result of the test must rest with the clinician if he is competent to judge. If however he does not feel competent to judge he should submit the clinical data and the result of the test to someone competent to correlate and decide on the diagnosis.

CHEMICAL AND EMPIRICAL TESTS.—These have already been mentioned in a previous chapter. Noguchi's butyric acid test (page 86), the cobra venom test (page 87) and Nonne's ammonium sulphate test (page 88) are all of importance especially when nervous symptoms exist. They usually are not specific for syphilis infections but occur when there is inflammation of the meninges. They are of value in giving added evidence and in some cases lead to examination that will clear up the diagnosis. The epiphanin test, based on the change in composition and drop-forming properties of blood serum under the influence of antibodies produced during disease, has been applied to the diagnosis of syphilis.

The method of Seiffert for the epiphanin test is to take 0.1 c. c. of a 10 per cent. dilution of the serum to 0.1 c. c. alcoholic extract of syphilitic liver; to this 1 c. c. of $\frac{N}{10}$ H_2SO_4 , and 1 c. c. of barium hydroxide of such concentration as will just neutralize the acid are added slowly. When one drop of an alcoholic solution of phenolphthalein is added, serum from a syphilitic turns red while in serum from a nonsyphilitic no tint is produced. By some this reaction is regarded of great value.

ALLERGY OR HYPERSENSIBILITY TESTS.—Allergy tests for diagnosis of various diseases have already been described. Various

tests have been tried for the diagnosis of syphilis. Meirowsky, Wolff-Eisner, Neisser and Bruck and others applied extracts of syphilitic chancre or foetal liver but these extracts were found not to be specific. Noguchi¹ with the development of methods for cultivating *Spirochæta pallida* was able to make new application of allergy in a cutaneous reaction in syphilis. The material for cutaneous injection he calls luetin. Luetin consists of the culture medium containing a heavy growth of pallida (see page 267), ground in a sterile mortar and diluted until perfectly emulsified. The emulsion is heated to 60° C. for one hour and 0.5 per cent. phenol added. It contains 40 to 60 pallida per field under the dark-field microscope.

To make the test the skin of both upper arms is cleaned. With a fine hypodermic needle a drop (0.05 c. c.) of luetin is injected intradermically in the left arm and into the skin of the right arm there is injected a similar amount of a control suspension consisting of the ground-up carbolyzed medium without pallida. Separate syringes should be used for the two suspensions. At the site of injection a small white spot is formed at the time of the injection.

The reactions produced vary. In normal individuals after 24 hours on both arms there is a small erythematous area about the point of injection. This recedes and leaves no induration. In syphilitics the reaction is different. The skin of syphilitics is susceptible to traumatic irritations. Neisser calls this "Umstimmung" and believes it to be due to a pathological condition of the skin. For this reason in syphilitics there is some degree of reaction about the control injection. Around the site of injection on the left arm the reaction is more severe. A papule five to ten millimeters may form in 24 to 48 hours. This is followed by induration and inflammation which usually gradually recedes but may go on to pustule formation. This pustule may break down when healing without scar formation occurs. This may require one to two weeks. The patient in all cases should be kept under observation for ten days as late reactions do occur. The test undoubtedly is of great value especially in the later stages of the disease. In the primary

¹Noguchi: J. Exper. M., 1911, XIV, 558, and Münch. med. Wehnschr., 1911, LVIII, 2372.

and secondary stages in patients not under treatment reactions frequently do not occur but may be present if the patient is under treatment. In tertiary, latent and inherited syphilis it occurs in most cases and here finds especial application for it is in these cases that frequently negative Wassermann reactions are observed. From Noguchi's experiments it is evident that the allergic condition does not persist long after the spirochætæ are eradicated by proper treatment.

Immunity and Specific Therapy.—The formerly accepted idea that recovery from syphilis confers lasting immunity can no longer be accepted. Experimental inoculation of apes has shown that new infections are possible. By our more recent tests the persistence of syphilis has been fairly well established. The law of Colle that when a mother free from syphilitic symptoms gives birth to a syphilitic child, the mother is immune but the child is capable of giving syphilis to others, and Profeta's law that a mother with syphilis may give birth to a child without taint are now refuted by some. These long accepted statements are not in accordance with the results of the Wassermann test. Whatever view we may take, syphilitic infection does produce changes that indicate the existence of processes which are a part of immunization. When reinfection occurs the course of the disease differs from that of the first infection.

Artificial immunization has not been successful. Metchnikoff, Roux and others have tried active immunization with attenuated cultures and Finger, Landsteiner and others have attempted passive immunization. The methods have no protective or curative value.

Chemotherapy.—Specific therapy for the treatment of syphilis has long been practiced. Syphilis is not a self-limited disease nor is it a disease in which nature only needs to be supported, but it is an infection for the cure of which *Spirochæta pallida* must be disposed of. This mercury has apparently been able to do. It banishes pallida from local lesions, renders the Wassermann reaction negative and causes the disappearance of outward manifestations of the disease. To get the full benefits of the drug it must be administered over a long period of

time and even after all symptoms and signs of the infection have disappeared. This probably more than anything else has been responsible for the incomplete treatment of syphilis with mercury. Patients often tire of the treatment especially when they are well and physicians frequently do not follow up their patients as they should. But incomplete treatment is not the only cause of failure for at times during mercury treatment new symptoms appear. In some patients mercury has no effect whatever and in others years after apparent cure new lesions appear especially in some parts of the nervous system. In some cases it is impossible to reach all of the spirochætæ and it is for this reason that potassium iodide is usually given during the course of the treatment. Iodides have further uses especially in relieving the periosteal pains and in affections of the nervous system.

In researches on trypanosomes Ehrlich observed that if the germicide is injected in a dose insufficient to kill all the organisms, there develops a strain of trypanosomes that are resistant to the germicide while in the body but susceptible in the test tube. In a previous chapter it has been stated that Ehrlich attempted to produce substances that have such marked affinity for the micro-organisms that they will combine only little with the body cells and are so toxic for the micro-organisms that they will kill all of them at the first injection (see page 134). The substances he found most efficient in trypanosomiasis belong to the groups of arsenical preparations, certain dyes as trypan red, trypan blue and trypan violet and certain basic dyes as methyl violet, pararufuchsin, and so on. Of many preparations, the six hundred and sixth (606, dichlorhydrate of dioxydiamino-arsenobenzol, salvarsan) he regarded as fulfilling the requirements for trypanosomiasis. From further experiments he concluded to try its efficiency in the cure of syphilis. This he did first by treating syphilis-infected rabbits and obtained complete cures without relapses. After this he tried it in human syphilis and when he believed he had found it effective and safe, he placed the preparation at the disposal of selected workers so that its efficiency, dosage, method of administration, indications, contra-indications and limitations

might be determined rapidly and before exploitation of the public should occur. In one year statistics were obtained on thousands of cases and salvarsan was accorded the distinction of being the most efficient specific remedy for the treatment of syphilis. Unfortunately however syphilis does not produce all of its ravages in one year; the disease manifests itself after years of quiescence and later results reported are not as conclusive as were the first ones.

The chemical salvarsan is a bright yellow powder containing theoretically 34.15 per cent. of arsenic. It is easily soluble in water, methyl alcohol and glycerin. Because of the readiness with which it undergoes oxidation and changes to a highly toxic product, it is put up in bottles from which the air has been removed or replaced by an indifferent gas. A new preparation of salvarsan, known as neosalvarsan and bearing the laboratory number 914 has been prepared. In this preparation the basis of salvarsan is not combined with hydrochloric acid but with sodium formaldehydsulphoxylate. This is a neutral yellow powder dissolving readily in water, less toxic than salvarsan, 1.5 grams of neosalvarsan corresponding to 1 gram of salvarsan. It is not necessary to alkalinize the aqueous solution. It is claimed to be as potent and better adapted for intramuscular injection.

For administration salvarsan was first used in aqueous solution but because the acid solution is too painful the solution is made alkaline by the addition of sodium hydroxide. The method of preparing the solution varies but the following one is generally adopted when salvarsan is to be injected intravenously. The salvarsan to be injected is put into a 500 c. c. bottle or flask containing some glass beads and then 30 to 40 c. c. of chemically pure physiological (0.9 per cent.) salt solution is added and the salvarsan dissolved by vigorous shaking. After solution of the salvarsan 0.19 c. c. of a 15 per cent. solution of sodium hydroxide is added for every 0.1 gram of salvarsan. A precipitate is formed which however dissolves on shaking. This is diluted with salt solution in amount sufficient so that every 50 c. c. of the final solution shall contain 0.1 grams of salvarsan. All of the solutions and containers should be sterile and kept so.

For intramuscular injection the salvarsan solution must be more

concentrated as only small amounts of fluid can be injected. Solutions that have been favorably received are those made in 4 to 10 c. c. of 10 per cent. iodopin, or those made in neutral oil.

Administration of salvarsan must be done under aseptic conditions and may be either intravenous, subcutaneous or intramuscular. For intravenous injection the solution is warmed and the tube and needle leading from the infusion bottle freed from air, that is filled with sterile salt solution. The needle need not be large. The solution should be put in slowly. The patient must be in bed and remain there for several hours at least. Before injecting salvarsan the administrator should be sure the vein has been entered and that there is no leakage into the tissues. Intramuscular injections are usually made into the buttocks or back and only small amounts of fluid are injected. Of the different methods the intravenous is to be preferred as by intramuscular and subcutaneous injections abscesses are frequently produced.

The dosage and manner of injection as planned by Ehrlich were such that one injection should kill all of the spirochætæ. This was done to conform to his idea of "therapia magna sterilisans." It was soon found however that the drug may be markedly poisonous to the patient and that salvarsan caused the death of a number of individuals. Naturally then the dose was decreased and Ehrlich's intention destroyed. If the maximal dose the patient can tolerate is less than the minimal dose that will kill all organisms present then we may again get drug-fastness in pallida. To get the best results and still not injure the patient all sizes of doses and reinjections of salvarsan have been proposed. It is impossible to give all of these methods. The general consensus of opinion is that enough salvarsan cannot be given at one time to kill all of the spirochætæ and that therefore several smaller doses must be injected. In the conservative treatment of syphilis with salvarsan, first every day or every other day hypodermic injections of mercury are given over a period of two weeks, then at intervals of three to four days 0.3 grams of salvarsan is given for four doses, and then at weekly intervals two injections of 0.5 grams of salvarsan are given. From this time on hypodermic injections of mercury should be given twice weekly for at least six months with occasional rests. Then if the Wassermann reaction is negative, injections are discontinued

and Wassermann tests made from time to time. If they remain negative for one year after the cessation of treatment and symptoms do not reappear the case is usually considered cured. In the intensive method daily intravenous injections of large doses even up to 1.0 gram are made for one week. Then mercury injections are given as in the other method. When neosalvarsan is used the doses are one and one-half times those of salvarsan. LaFetra advises the use of repeated injections of 0.01 grams of salvarsan per kilogram of body weight for children.

The reactions due to salvarsan injections come on after about two hours. They are headache, fullness in the head, nausea, vomiting, sleeplessness and slight fever. These symptoms are usually avoided when the conservative method is followed. Finger who has carefully observed large numbers of patients that have received salvarsan states that in a majority of cases no matter what the method of the application of the remedy may be there is rigor, fever, general indisposition, vertigo, headache, nausea, vomiting, loss of appetite, oppression of the heart and respiratory organs, restlessness, and so on. In some cases there is early cyanosis and edema of the face, vomiting, diarrhœa, dyspnea, spasm of the diaphragm and other muscles and collapse. These symptoms are regarded as definitely those of arsenical poisoning.

A few weeks after administration of salvarsan other nervous symptoms may come on. The clinical picture in these cases varies. There may be headache, exhaustion, loss of appetite and disturbance of nutrition and extreme forgetfulness. In other cases there is deafness, in some monoplegia, epileptiform attacks, optic neuritis, breaking down of infected glands as in tuberculosis, encephalitis, and so on.

The beneficial results of salvarsan have usually been judged by the disappearance of rashes, mucous patches and so on. Apparently greater benefits are derived from the treatment in some cases than in others and in some stages than in others. Some of the unfavorable symptoms and signs experienced after treatment have been attributed to the liberation of endotoxins from the killed spirochætæ, but this is not entirely agreed

to especially so since the symptoms and signs observed are not unlike those of arsenical poisoning.

It cannot be stated at this time that salvarsan is more specific or efficient than mercury. It will take from twenty to thirty years to decide that. It is known what mercury and the iodides can do and with our improved methods and preparations of insoluble salts of mercury still greater benefits are to be looked for. When the physician is compelled to use salvarsan he should not depend on it alone. The idea of Ehrlich's sterilizing dose has been defeated because with salvarsan this is unsafe. It is to be hoped that the remedy will not be cast aside for a time as was tuberculin but that it will be used honestly and conscientiously when indicated.

Prophylaxis.—This in most cases is the same as for all venereal diseases. Continence is the greatest factor but for individuals who cannot be persuaded to this, instruction in the protective value of personal cleanliness, disinfection after exposure and the indulgence in coitus only when not under the influence of liquor are logical and useful. Thorough washing and cleansing of the urethra with a 1 to 200 silver nitrate solution and the use of a 30 per cent. calomel ointment for outer application certainly are of value. For this instruction is necessary. To make the disease one that must be reported so that the proper authorities can insist on treatment and take precautions to prevent further infection would undoubtedly be of value for preventive purposes.

INFECTIONS WITH MALARIA PARASITES.

Malaria is an infectious disease characterized by paroxysms of intermittent fever. It is often confused with other diseases and unfortunately many cases of irregular fever are wrongly diagnosed as malarial. Because of this the disease is mentioned here, as well as to remind the clinician that there are definite means of diagnosing malaria and that quinin treatment is so specific that it may be used as a diagnostic agent.

The disease malaria is caused by protozoa belonging to the plasmodia. They were found in the circulating blood of ma-

laria patients by Laveran in 1880. Different types of malaria are recognized. The fever may occur regularly, in the tertian type every other day and in the quartan there are two fever-free days and then one day with fever. Then there is a type in which there is more irregular remittent or continued fever. This is known as the æstivo-autumnal form. These primary forms of malaria are caused by different species of plasmodia. The tertian type is caused by *Plasmodium vivax*, the quartan type by *Plasmodium malarie* and the æstivo-autumnal by *Plasmodium præcox*. In some cases infection is not with a single species and at times there may be new infections with the same type. In these cases there may be fever on irregular days; that is there may be two fever days and one free day and so on, and still the species belong to one of the types producing paroxysms at regular intervals.

All of the malaria parasites have two cycles—a sexual cycle and an asexual cycle. Of these the sexual cycle occurs in man and the asexual cycle occurs in a certain genus of mosquito known as the anopheles. In order that a person be infected it is necessary that a mosquito of the genus anopheles be infected by biting a malaria-infected person, that the parasite go through its sexual cycle in the mosquito and that the mosquito containing the malaria parasite in its proper stage of development bite and infect the person. A certain period of time must elapse after infection to produce the disease in man. It is impossible for man to contract the disease directly from man. The disease is not spread by bad water. To have the disease there must be infected anopheles. To breed these there must be standing water and for the mosquito to become infected it must bite a person with malaria.

Differential Specific Diagnosis.—It has already been stated that malaria is frequently diagnosed when it does not exist. Many forms of intermittent and irregular fever are wrongly diagnosed as malaria.

CLINICAL.—In typical cases this is easy. The regularly recurring febrile attacks and lack of symptoms between paroxysms are characteristic. Usually fever is preceded by a chill and followed by profuse perspiration. As the disease goes on the

patient becomes anemic, the hæmoglobin content is reduced to 40 or 50 per cent. there is enlargement of the spleen and cachexia follows.

The best diagnosis is one based on the blood examination for malaria parasites but when this is not possible a clinical test will usually give a good diagnosis. An intermittent fever not yielding to quinin is not malarial. To distinguish malaria from most infections a leucocyte count is of value as usually in malaria there is no leucocytosis while in other infections there is.

MICROSCOPICAL DIAGNOSIS.—This is most reliable. For microscopical examination blood should be examined fresh and on properly stained slides. When there are few parasites the organisms are more easily found in stained smears. When there are very few parasites a thick drop of blood is put on the slide, dried in the air and then the hæmoglobin is washed out with water. After this the specimen is stained in the usual manner. For staining Romanowski's, Jenner's, Hasting's or any of the polychrome methylene blue-eosin stains may be used. Of all methods, examination of the fresh unfixed blood is the most satisfactory. To examine a fresh specimen the drop of blood is placed on the slide and a cover glass lightly put on. The edge of the cover glass may be sealed with vaseline or olive oil. All specimens should be examined under the oil immersion lens.

The parasites should be looked for in the red blood cells although they may also be recognized outside of the red corpuscles. The organisms are stained blue and the chromatin red with the polychrome methylene blue-eosin stains.

For differentiation between tertian and quartan infection various points are of assistance. The quartan is less amœboid, more refractive and smaller. Its pigment is coarsely granular, lies in the periphery and is more quiet. The corpuscles containing the organisms are smaller than normal and are crenated. When segmentation occurs fewer segments are formed, six to twelve in number. The organisms causing æstivo-autumnal malaria are recognized especially by the crescents. These are longer than the red blood cells and form a fringe for the degener-

ated corpuscle. In the fresh specimen the crescent changes its shape becoming oval, circular and again crescentic. In this infection pigmented granules are frequently found in phagocytic cells.

CULTURAL METHODS IN DIAGNOSIS.—Bass¹ has succeeded in cultivating malaria plasmodia. So far growth has been attained only in the red blood cells of man. The organisms grow best at a temperature of 40° C. and digest the red corpuscles. Organisms of all three species have been cultivated but in cultures only the asexual cycle has been observed. It is to be hoped that the cultivation method may prove of value in diagnosis and treatment of malaria.

Immunity and Specific Therapy.—For some time a natural immunity was claimed for natives in malarial countries. Now however it has been established that in these countries the children have malaria and the adults have an acquired immunity. When there is interruption of malarial infection in such countries susceptible adult natives are found. Moreover acquired immunity to one type of parasite does not protect against the others. As far as we are concerned there probably is no real immunity either natural or acquired. Active and passive immunization are of no avail in this disease. For years however a specific for malarial infection has been known. In quinin we possess a specific remedy. Formerly 20 to 30 grains of quinin were given daily in divided doses. Now large doses are given especially just before the paroxysms occur. At first large doses are given and then smaller ones. In æstivo-autumnal malaria quinin may be given intravenously. Treatment should extend at least over a period of a month and not be left to the discretion of the patient for drug-fastness may result in the plasmodia.

Prophylaxis.—As has already been stated the infection goes from man to anopheles and again to man. The anopheles differ from the ordinary mosquito in that the eggs are not glued together, the larval forms in water lie parallel to the surface, the body of the mosquito when sitting against a surface is straight and held at an angle of about 45 degrees, the wings

¹Bass: J. Am. M. Ass., 1911, 1534; J. Am. M. Ass., 1912, LIX, 937.

are spotted and the projections from the front of the head are of the same length.

The anopheles breed in water, the adult usually does not fly far (one-half mile) from its breeding place but may be carried great distances by streams, conveyances and successive breedings; they avoid high winds, bite especially at night or after sundown, and only the females suck blood, the males obtaining their food especially from fruits. From the egg to the adult requires from 15 to 25 days and the adults live for many months.

The development of malaria plasmodia in the mosquito requires from 10 to 14 days during the heat of summer and 14 to 18 days in the fall and spring. In man 10 to 12 days are required after the bite of an infected mosquito for the appearance of symptoms.

For the prevention of malaria recognition of infected persons and extermination of anopheles are necessary. In regions where anopheles mosquitoes exist persons should be protected by screening, and all swamps, dumps, wagon ruts and so on should be drained. When this is impossible oil should be poured on the surface of the water. The efficiency of such methods has been amply proven. Clove, lavender or citronella oils when applied to the body prevent biting by mosquitoes; burning of pyrethrum powder or even smoke from burning papers drives mosquitoes out of the room. Quinin taken in doses of 15 grains every 10 to 11 days while in anopheles-infested localities is of great value in prophylaxis.

BIBLIOGRAPHY.

- ABEL AND FORD, 15
 ALBERT AND MENDENHALL, 213
 ARLOING AND COURMONT, 182
 ARMS, 202
 ARONSON, 148
 ATKINSON, 122
 AUER, 132
 AUSTRIAN, 209
 BABES, 257
 BABES AND LEPP, 12, 115, 258
 BAGINSKY, 149
 BAIL, 1
 BALDWIN, 76
 BANZHAF, 122, 236
 BASS, 283
 BEHRING, 12, 25, 115, 190, 198, 228
 BEHRING AND KITASATO, 12, 15, 115, 234, 244
 BEHRING AND KNORR, 12, 116
 BEHRING AND WERNICKE, 13, 234
 BELFANTI AND CARBONE, 59, 122
 BERGERON, 180
 BESREDKA, 28
 BIER, 98
 BOLDUAN, 76, 77
 BOLTON, 117
 BOOKER, 216
 BORDET, 20, 25, 52, 56-60
 BORDET AND GENGOU, 59-65, 163, 268
 BOSTOCK, 259
 BRIEGER, 12
 BRION AND KAYSER, 205
 BRONFENBRENNER AND NOGUCHI, 22
 BROWN, 194
 BROUGHTON-ALCOCK, 213
 BRUCK, 151
 BUCHNER, 12, 20, 25, 115
 BULLOCH AND ATKIN, 28
 CALKINS, 248
 CALMETTE, 83, 187, 192
 CARLE AND RATTONE, 242
 CASTELLANI, 212
 CATLIN, SCOTT AND DAY, 242
 CHAMBERLAND, 12
 CHANTEMESSE, 209, 213, 214, 215
 CHAUVEAU, 10
 CLARK, 108, 111
 COLE, 76, 81, 157, 159
 COLLIGNON AND PILOD, 170
 COUNCILMAN, 248
 COURMONT AND DOR, 190
 COWIE AND CHAPIN, 28
 COYNE AND AUICHE, 224
 CRAIG, 271
 CRAIG AND NICHOLS, 272
 CUSHING AND SLADEN, 174
 DEAN, 28
 DENIS, 127
 DENY, 191, 193
 DENYS AND LECLEF, 25, 65, 147
 DETRI, 60, 268
 DEUTSCH, 29
 DIEUDONNE, 122
 DIXON, 198
 DOCHEZ, 159
 DOGANOFF, 187
 DOPTER, 221
 DORR, 29, 227
 VON DRIGALSKI, 206
 VON DRIGALSKI AND CONRADI, 207
 DUNBAR, 259
 DUNHAM AND ELSEY, 170
 DUVAL AND BASSETT, 216
 DUVAL AND VEDDER, 216
 EHRLICH, 13-24, 25, 29, 56, 119, 126, 135, 276
 EHRLICH AND MARSHALL, 21
 ELLERMANN AND ERLANDSEN, 186
 ELLIOTSON, 259
 ENGEL AND BAUER, 186

- ESCHERICH, 149
 FEHLEISEN, 143
 FERRAN, 224, 227
 FISHBERG, 89
 FINGER, 275, 279
 FLEXNER, 170, 171, 216, 223
 FLEXNER AND JOBLING, 172, 173
 FLEXNER AND NOGUCHI, 90, 261
 FLEURING, 142
 FLOYD AND BARKER, 209
 FLOYD AND LUCAS, 161
 FOA AND BONOME, 12
 FODOR, 12, 25, 115
 FORD, 206
 FORD AND ABEL, 15
 FRÄNKEL, 12, 155, 158, 163, 234
 FRIEDLÄNDER, 162
 FRIEDMANN, 198
 FROSCH, 90
 FULLERTON, 88
 GABRITCHEWSKY, 146
 GAERTNER, 207
 GAY AND SOUTHARD, 130
 GIBSON, 122, 236
 GILCHRIST, 142
 GREENFIELD, 86
 GRUBER AND DURHAM, 45
 GRÜNBAUM, 45
 GRÜNBAUM AND HUME, 207
 GUANIERI, 248
 HAECKEL, 11
 HAFFKINE, 228, 229
 HAGEMAN, 184
 HAMILTON, 108
 HAMMAN AND WOLMAN, 187, 194
 HARRIS, 147, 257
 HECKER, 21
 HEILNER, 132
 HEKTOEN, 27
 HELMAN, 202
 HERRICOURT, 83
 HERRICOURT AND RICHET, 12, 115
 HEUBNER, 129
 HEWLETT AND NANKIVELL, 235
 HIRSCHFELDER, 158
 HISS, 36, 119
 HISS AND RUSSELL, 217
 HISS AND ZINSSER, 80, 136, 161
 HOLZMAN, 87
 HORDER, 147
 HOUSTON AND RANKIN, 170
 HÜHNE AND NEUFELD, 28
 INABA, 163
 IRONS, 84, 151
 JAEGER, 171
 JENNER, 82, 250
 JESSEN, 191
 JEZ, 215
 JOCHMAN, 163, 171, 172, 191
 JOBLING, 172, 249
 JORDAN, 248
 JÜRGENS, 206
 KABESHIMA, 212
 KALNING, 202
 KEIDEL AND HURWITZ, 269
 KENDALL AND WALKER, 206, 219
 KITASATO, 229, 242
 KLEBS, 10, 192, 231
 KLEMPERER, 158, 181
 KLENCKE, 175
 KLIMENKO, 163
 KNOX AND SCHORER, 217, 218, 220
 KNOX AND SLADEN, 174
 KOCHER, 247
 KOLLE, 228, 230
 KOLLE AND WASSERMANN, 171, 172
 KOCH, 10, 31, 32, 82, 148, 175, 182, 187,
 191, 192, 194, 226
 KRAS, 247
 KRAUSE, R., 52, 163, 227
 KRUSE, 216
 KUTSCHER, 170
 LAMAR, 161
 LANDSTEINER, 275
 LANGMAN, 192
 LAVERAN, 281
 LEARY, 107
 LEISHMAN, 26, 65, 66
 LEVADITI AND INMAN, 27
 LEVY-VALENSI, 88
 VON LINGELSHEIM, 170
 LIPIERRE, 171
 LOEFFLER, 31, 90, 231
 LOEFFLER AND SCHÜTZ, 200
 LOEHLEIN, 28
 LOESCH, 216
 LONDON, 20, 152
 LUCAS, 169

- LUCAS AND AMOSS, 221
LUSTGARTEN, 263
LUSTIG AND GALEOTTI, 230
LUSTIG AND MARKL, 230
MAGENDIE, 82
MALLORY AND HORNER (AND HENDERSON), 163
MALVOZ, 60
MANICATIDE, 164
MANOUKHINE, 161
MANTOUX AND ROUX, 84
MARAGLIANO, 193, 199
MARIE, 257
MARMOREK, 147, 199
MARTINI AND LENTZ, 217
MARTIN AND GRANCHER, 190
MARX, 23
MCCLINTOCK AND KING, 126, 240
MC CONKEY AND HILL, 217
MCFAYDEN, 190
MEAKINS, 115, 170
MEIROWSKY, 274
MELTZER, 247
MENDEL, 187
MENZER, 149
METCHNIKOFF, 11, 20, 24, 56, 119, 228, 263, 275
METCHNIKOFF AND BESREDKA, 212
MILLER AND GRAEF, 205
MORESCHI, 60
MORO, 84, 187
MORGENROTH, 119, 161
MORLAND, 186
MOSS, 76, 77, 79, 81, 133
MUCH, 87
MUIR AND MARTIN, 27, 28
MÜHLENS AND HOFFMANN, 267
MÜLLER, 20
NEGRI, 89, 254
NEISSER, 150
NEISSER AND BRUCK, 84, 268, 274
NEISSER AND SACHS, 60
NEPOROSCHNY, 199
NEUFELD, 27, 159, 190
NEUFELD AND RIMPAU, 26-28, 65-82, 148
NEUFELD AND TOEPFER, 28
NICOLAIR, 242
NOEGERATH AND STAEHELEIN, 266
NOGUCHI, 61-65, 84, 86, 169, 266, 268, 274
NOGUCHI AND BRONFENBRENNER, 21, 62, 65, 268
NONNE, 88
NUTTALL, 12, 25, 115
OGSTON, 143
OTTO, 83
PARK, 76, 238
PARK AND WILLIAMS, 235
PASTEUR, 10, 31, 100, 155, 256
PEARSON AND GILLILAND, 190, 198
PETERSEN, 80
PETRUSCHKY, 148, 235
PFEIFFER, 23, 45, 56, 165
PFEIFFER AND ISSAEFF, 55
PFEIFFER AND KOLLE, 211
PHYSSALIX AND BERTRAND, 13
VON PIRQUET, 185, 252
VON PIRQUET AND SCHICK, 83, 127, 130, 149
PORTIER, 83
POTTER, 76
RAVENAL, 242
RENDU, 240
RICHARDS, PEABODY AND CANAVAN, 220
RICHTER, 83
ROSENAU AND ANDERSON, 83, 129, 132
ROSENBACH, 143
ROSENBERGER, 180
ROSENOW, 113, 157, 159
ROSS AND JOHNSON, 146
ROSTOSKI, 209
ROUX, 12, 192, 228, 263, 275
ROUX AND YERSIN, 12, 116
RUEDIGER, 108
SABOURAUD, 142
SACHS, 21
SADLER, 212
SAHLI, 193
SALMON AND SMITH, 12
SAUERBEK, 29
SAVTCHENKO, 28
SCHICK, 149
SCHAUDINN AND HOFFMANN, 263
SCHERESCHEWSKY, 267
SCHORER, 69, 77, 108, 110
SCHOTTMÜLLER, 157, 204
SCHUT, 175

- DE SCHWEINITZ, 190, 202
SEIFFERT, 273
SELLARD AND JEANS, 70, 81
SHERRINGTON, 6
SHIGA, 216, 221, 223
SIEGEL, 263
SIMON, 76
SIMON, LAMAR AND BISPHAM, 79, 81
SMITH, 83
SOPHIAN, 171
SPENGLER, 163, 192
STERNBERG, 155
STRAUS, 21, 201
STRONG, 82, 230
SZONTAGH, 210
TAVEL, 148, 214
TCHISTORVITSCH, 52
TEICHMAN, 186
TERNUNCHI, 21
TIZZONI AND CENTANNI, 258
TORREY AND ROGERS, 153
TRUDEAU, 190, 192
TUNNICLIFF, 107
UHLENHUTH, 53
VAILLARD AND DOPTER, 223
VALLEE, 186
VAN DER VELDE, 147
VEDDER AND DUVAL, 216
VILLEMIN, 180
VINCENT, 171
WADSWORTH, 158
WALKER, 71, 72, 76, 77, 79
WALTERS AND EATON, 212, 213
WASSERMANN, 23, 52, 59-65, 117, 268
WASSERMANN AND BRUCK, 60
WEAVER AND TUNNICLIFF, 148
WEICHSELBAUM, 155, 167
WEIGERT, 14
WEIL, 87, 130
WELCH, 6, 10
WERNICKE, 126
WHEELER, 115
WIDAL, 45, 46
WOLF, 52
WOLFF-EISNER, 84, 186, 187, 274
WOLLSTEIN, 38, 163, 165, 166, 211, 213
WRIGHT, 27, 65-82, 102, 103-112, 115, 141, 193, 211
WRIGHT AND DOUGLAS, 25-28, 43, 65-82, 96, 103-112, 148
WRIGHT AND RIED, 26
WYSSOKOWITSCH, 6
YERSIN, 229
ZINKE, 253

INDEX.

A

- Acne vulgaris, 141
Active serum, 20, 24, 62, 118
Agglutination for diagnosis of etiological factor in disease, 40, 44-51
 for identification of bacteria, 39
 Gruber-Widal reaction, 45, 207
 interpretation of test, 48
 in typhoid fever, 207-209
 macroscopic test, 47
 microscopic test, 47
 Widal reaction, 45, 207
Agglutinins, 18, 40-51, 97, 111, 118, 204, 208
 in diagnosis, 19, 39, 40, 44-51
 value of, in immunization, 97, 118, 220
Aggressins, 1, 29
Albumin test of sputum, 88
Alexins, 12, 19, 22, 115
Allergins, 131
Allergy in diagnosis, 82-85
 method for test, 84
 reaction, 84
Amboceptor, 19-24
 in lysin test, 55-59
 for diagnosis of syphilis, 63, 269
Ammonium sulphate test, 88-273
Anaphylaxis, 82-85, 126-134, 241
Anaphylactine, 130
Animal inoculation for diagnosis, 39
Anopheles, 281
Antibacterial sera, 19, 118, 120, 124
Antibodies, distribution of, 23
 group, 18, 24, 204, 208, 220
 loss of, 23
 production of, 14, 17-25, 39, 63, 94-134
 prophylactic value of, 95
 specific, 24, 204
 value of, in diagnosis, 39
 value of, in immunization and immunity, 96
 value of, in treatment, 95
Anticomplement, 23, 119, 268
Antidysenteric serum, 221
Antiferment, 23
Antigen, 20, 59, 62, 99, 268
Antigonococcic serum, 153
Antimeningococcic serum, 172
Antiplague serum, 230
Antipneumococcic serum, 159
Antiserum, 97, 115-137
 administration of, 125, 173, 237
 indications for, 123
 local application of, 98, 126, 240, 247
 mixed, 99

- Antistaphylococcic serum, 142
- Antistreptococcic serum, 147
- Antitoxic globulins, 122, 236, 245
- Antitoxic sera, 117, 120, 124
 - diphtheria antitoxin, 235, 240
 - dysentery antitoxin, 221
 - hay fever serum, 260
 - tetanus antitoxin, 244, 247
- Antitoxin, 18, 97, 117, 124
- Antitoxin unit, 120, 236, 245
- Antityphoid serum, 214
- Antivenin, 13
- Arsenobenzol (*see* salvarsan), 135, 276
- Articular rheumatism, 144, 147, 149
- Atreptic theory, 29
- Attenuation, use of, in immunization, 9
- Autogenous vaccine, 104
- Autoserotherapy, 137
- Autumnal fever, 259-260
- Avirulence, 30

B

- Bacillus (*see* also Bacterium)
- Bacillus, coli communis, 68, 96, 225-226
 - Differential diagnosis of, 225
 - Immunity and specific therapy, 226
 - Infection with, 225
- Bacillus enteritides, 203
- Bacillus, Klebs-Loeffler, 231-242
- Bacillus paratyphosus, 204, 207
- Bacillus pestis, 97, 229-230
 - Differential diagnosis of, 229
 - Immunity and specific therapy, 229-230
 - Infections with, 229
 - Prophylaxis, 230
- Bacillus pyocyaneus, 68
- Bacillus typhosus, 68, 96, 204-216
 - Differential diagnosis of, 204-210
 - allergy tests, 209-210
 - bacteriological, 205-207
 - blood cultures in, 205
 - clinical, 205
 - feces, 206
 - roseolar spots, 205
 - serum (agglutination), 207-208
 - Immunity and specific therapy, 210-215
 - active immunization, 211, 215
 - passive immunization, 214
 - serum therapy, 214
 - vaccine therapy and vaccination against, 211, 215
 - Infections with, 204
 - Prophylaxis, 215
- Bacteria, cultivation of, 36
 - local application of, in infection, 98
 - identification and isolation of, 35-39
- Bacterial toxins, 4, 10, 12, 15, 117
- Bacterial products in immunization, 112
- Bactericidal sera, 19, 118, 124
- Bacterins, 9, 100, 214
- Bacteriolysins, 19-24, 111, 118

- Bacteriolysis in diagnosis, 55-59
 - in treatment, 96, 118-120
- Bacteriolytic amboceptors, 16, 19, 55-59, 118
- Bacteriolytic tests, 55-59
- Bacteriotropic theory of immunity, 24-29
- Bacteriotropin, 26
- Bacterium diphtheriæ, 91, 97, 231-242
 - Differential diagnosis of, 231-234
 - bacteriological, 232
 - clinical, 231
 - serum, 234
 - Immunity and specific therapy, 234-242
 - active immunization, 234, 242
 - antitoxin, 235
 - passive immunization, 235, 242
 - serum therapy, 235
 - vaccine, 234
 - Infections with, 231
 - Prophylaxis, 241
 - Toxin, 235
- Bacterium dysenteriae, 96, 216-225
 - Differential diagnosis of, 218-221
 - bacteriological, 219
 - clinical, 218
 - serum, 220
 - Immunity and specific therapy, 220-225
 - active immunization, 221
 - passive immunization, 221
 - serum therapy, 221
 - vaccination and serum therapy, 221, 224
 - Infections with, 216
 - Prophylaxis, 224
 - Types of, 217, 223
- Bacterium influenzae, 87, 164-167
 - Differential diagnosis of, 165-166
 - Immunity and specific therapy, 166-167
 - Infections with, 164
 - Prophylaxis, 167
- Bacterium mallei, 200-203
 - Differential diagnosis, 200-202
 - Immunity and specific therapy, 202
 - Infections with, 200
 - Mallein in (hypersensibility), 202
 - Prophylaxis, 203
- Bacterium pneumoniae, 162
 - Diagnosis and specific therapy, 162
- Bacterium tetani, 242-248
 - Antitoxin, 245
 - Differential diagnosis of, 243-244
 - Immunity and specific therapy, 244-248
 - active immunization and vaccine therapy, 244
 - passive immunization and serum therapy, 244
 - Infections with, 243
 - Prophylaxis, 246, 247
 - Toxin, 245
- Bacterium tuberculosis, 70, 74, 87, 91, 175-200
 - Albumin test of sputum, 88
 - Allergy (tuberculin tests), 182-190
 - cutaneous test (von Pirquet), 185
 - intracutaneous test, 187
 - ocular (Calmette), 187

- Bacterium tuberculosis—Cont'd
 percutaneous test, 187
 subcutaneous test, 184
 value of various tests, 188
Bacteriological diagnosis, 176-181
 blood, 180
 exudates, 179
 feces, 180
 operative material, 179
 sputum, 177
 urine, 179
Differential diagnosis, 176-190
Immunity and specific therapy, 189-200
 active immunization, 190-199
 by means of avirulent cultures, 198
 by Dixon's method, 198
 by Friedmann's method, 198
 with tuberculins, 191-199
 with vaccines, 198
 Passive immunization and serum therapy, 199
 vaccine therapy (*see also* active immunization), 198
Infections with, 175
Prophylaxis, 190
Tuberculin, 191-199
 administration of, 193
 dosage of, 194
 duration of treatment with, 197
 intervals between injections, 194
 selection of, 192
 sight of injection of, 197
Tuberculins, 191
Types of tubercle bacilli, 175
Bacterium xerosis, 34
Behring's law, 116
Blood cultures, 37, 205
Blood, determination of origin by precipitin test, 53
Blood for serum tests, 41-44
Blood, viscosity of, 97
Bordet and Gengou test, 59-65, 268-273
Bovine tuberculosis, 175
Bubonic plague (*see* *Bac. pestis*), 229-230
Butyric acid test, 86, 273

C

- Cerebrospinal fluid, antibodies in, 168
 how to obtain it, 169
Cerebrospinal meningitis (*Diplococcus intracellularis*), 167-174
Chemical tests in specific diagnosis, 85-89, 273
Chemical theory of immunity, 12
Chemotherapy, 134, 161, 275-280, 283
Cholera (*see* *vibrio cholerae*), 226-228
Cobra venom test, 87, 273
Complement, 19-24, 59-65, 119, 268-273
 absorption and fixation of, 39, 41, 59-65, 268-273
 how to obtain it for tests, 62, 269
Complementophore group, 22
Concentration of serum, 121-123
Conradi-Drigalski medium, 207
Copula, 20

Corpuscle suspension, 63
Cowpox, 250
Cytase, 11
Cytolytic antibody, 22, 63, 269
Cytophylic group, 22

D

Desmon, 20
Deviation and deflection of complement, 59-65, 268-273
Diagnostic value of agglutination reaction, 51, 208
 of bacteriolysis test, 59
 of fixation of complement test, 62, 272
 of laboratory tests, 34, 92, 138
 of precipitin test, 55
 of Wassermann test, 63, 272
Diphtheria (*see* *Bacterium diphtheriae*), 231-242
Diplococcus intracellularis, 167-174
 Differential diagnosis, 168-170
 bacteriological, 169
 chemical tests in, 169
 clinical, 168
 serum, 170
 Immunity and specific therapy, 170-174
 active immunization, 171, 174
 Flexner-Jobling serum, 172
 passive immunization, 172
 serum therapy, 172
 vaccine therapy, 171, 174
 Infections with, 167
 Lumbar puncture, 169
 Prophylaxis, 174
Diplococcus pneumoniae (*see* *Micrococcus pneumoniae*), 155-161
Drigalski-Conradi medium, 207
Dysentery (*see* *Bacterium dysenteriae*), 216-225

E

Ebert's bacillus (*see* *Bacillus typhosus*), 204-216
Ehrlich's 606, salvarsan, arsenobenzol, 135, 276
Ehrlich's side-chain theory, 13-24
Elimination of bacteria, 6
Empirical tests in diagnosis, 85-89
Emulsion of bacteria, 46, 52, 57, 62, 68-71
Emulsion of red blood corpuscles, 63
Endocarditis, 148
Endotoxin, 4, 29, 118
Epidemic cerebrospinal meningitis (*see* *Diplococcus intracellularis*), 162-174
Epidemic poliomyelitis, 90, 261
Epiphanin test, 273
Epitoxoid, 17
Erysipelas (*see also* *Strep. pyogenes*), 108, 143, 146, 148
Exanthematous fevers, 33, 89, 90
Exhaustion theory of immunity, 10
Extracellular toxins, 4, 15, 222, 235, 245
Extracts of bacteria in diagnosis, 51, 60, 82
 in treatment, 112
Extracts of leucocytes, 29, 119, 136, 161

F

- Farcy (*see* *Bacterium mallei*), 200-203
 Federal control of vaccines and sera, 99
 Filterable viruses, 33, 89-90
 Fixation of complement in diagnosis, 39, 41, 59-65
 in syphilis, 60, 268-273
 Fixator, 11, 20
 Fixed virus, hydrophobia, 256
 Fixing of smears for bacteriological examination, 35
 for opsonic index determination, 74
 Flexner serum, 172
 Fränkel's pneumococcus, 155-161
 Friedmann's treatment of tuberculosis, 198

G

- Gaertner's bacillus, 203
 Glanders (*see* *Bacterium mallei*), 200-203
 Globulins in sera, 121-123
 Glucosides, 15
 Gonococcus (*see* *Micrococcus gonorrhææ*), 150-155
 Gonorrhæa, 84, 150-155
 Group agglutinins, 18, 24, 204, 208, 220
 Gruber-Durham reaction (*see* agglutination), 45, 207
 Gruber-Widal reaction (*see* agglutination), 45, 207

H

- Hæmolysis, 22, 63, 270
 Hæmolytic amboceptor, 22, 63, 269
 Hæmolytic system, 63, 269
 Haptophore group, 16
 Hay fever, 259-260
 Causal factor of, 259
 Differential diagnosis of, 259
 Immunity and specific therapy, 259
 Pollantin, 260
 Hydrophobia (*see* rabies), 253-259
 Hyperæmia, 98
 Hypersensibility or hypersusceptibility to serum, 82-85, 126-134, 241
 to vaccine, 82-85, 106
 Humeral theory of immunity, 12

I

- Identification of bacteria, 35-39
 Immune sera, antibacterial, 19, 118, 120, 124
 anticholera, 227
 antidiphtheritic, 235, 240
 antidysenteric, 221
 antigonococcic, 153
 antiplague, 230
 antipneumococcic, 159
 antistaphylococcic, 142
 antistreptococcic, 147
 antitetanic, 244, 247
 antitoxic, 117, 120, 124
 antityphoid, 214

- Immune sera, antibacterial—Cont'd
dried, 123, 240, 247, 260
hay fever, 259-260
polyvalent, 147
Immunity, 8, 30, 94
acquired, 8, 94
active, 9, 12, 95, 100-115
artificial, 9, 94-137
duration of, 8, 10, 100
natural, 8, 94
passive, 9, 12, 95, 115-134, 136
theories of, 8-30, 97, 107, 119, 136, 161
Immunization, active, by means of bacterial cell proteids and products, 112
curative, 95
prophylactic, 95, 100
Immunizing substances, 10, 15, 100
Inactivation of serum, 20, 24, 62, 118, 269
Inclusion bodies, 89, 253, 255
Incubation period, 2
Infections, 1-7
Influenza (*see* *Bacterium influenzae*), 164-167
Inulin serum water, 157

K

- Kleb's-Loeffler bacillus (*see* *Bacterium diphtheriae*), 231-242
Koch's bacillus (*see* *Bacterium tuberculosis*), 175-200
Koch's canons or laws, 32
Koch's lymph (*see* tuberculin), 191-199
Koch's tuberculin (*see* tuberculin), 191-199

L

- Lapine, 251
Leistungskern, 13
Leucocidin, 140
Leucocytes in differential diagnosis, 90-92
in Metchnikoff's theory of immunity, 11
in opsonic index determinations, 66, 80
in treatment, 29, 119, 136, 161
Leucocytosis in infections, 90-92
Local application of antiserum, 28, 126, 240, 247
Locus minoris resistentiae, 3, 168
Loeffler's serum, 233
Lysins in diagnosis, 39, 40, 55-59
Pfeiffer's method, 56
test tube method, 57
Lysis in diagnosis, 55-59
in fixation of complement tests, 63, 270
in immune sera, 55
in normal sera, 37, 55
value of, in immunity, 96
Lyssa (*see* rabies), 253-259

M

- Malaria, 91, 280-284
Anopheles, 281, 284
Differential diagnosis, 281-283

- Malaria—Cont'd
 Immunity and specific therapy, 283
 Prophylaxis, 283
 Quinin in, 283, 284
 Mallein, 202
 Marmorek's serum, 147
 Measles (leucocytes in), 91
 Meningitis, acute epidemic (*see* *Diplococcus intracellularis*), 167-174
 tuberculosis, 168
 Menzer's serum, 129
 Mercury treatment in syphilis, 275
 effect on fixation of complement test, 275
 Metchnikoff's theory of immunity, 11
 Micrococcus gonorrhœæ, 68, 150-155
 Differential diagnosis of, 151-152
 Immunity and specific therapy, 152-155
 Infections with, 150
 Prophylaxis, 155, 280
 Serum therapy, 153
 Vaccine therapy, 152
 Micrococcus intracellularis (*see* *Diplococcus intracellularis*), 87, 167-174
 Micrococcus lancetatus (*see* *Micrococcus pneumoniae*), 155-161
 Micrococcus meningitidis (*see* *Diplococcus intracellularis*), 68, 91, 167-174
 Micrococcus pneumoniae, 68, 87, 91, 97, 155-161
 Chemotherapy in, 161
 Differential diagnosis of, 156-157
 Immunity and specific therapy, 157-161
 Infections with, 155
 Leucocytic extracts in treatment, 161
 Prophylaxis, 161
 Micrococcus pyogenes, 68, 91, 138-143
 Differential diagnosis, 139-140
 Immunity and specific therapy, 140-142
 Infections with, 138
 Prophylaxis, 142
 Microscopic identification of micro-organisms, 35
 Morvin, 202
 Moser's serum, 149

N

- Negative phase, 96, 102
 Negri bodies, 85, 253, 255
 Neosalvarsan, 277
 Noguchi butyric acid test, 86, 273
 fixation of complement test, 61, 268
 method of cultivating *Treponema pallidum*, 267
 Noxious retention theory of immunity, 10

O

- Opsonic index, 26, 40, 65-82, 107
 bacterial emulsions for, 68, 79
 in diagnosis, 40, 65-82
 in normal individuals, 76
 leucocytes for, 66, 80
 serum for, 41, 66
 slides for, 73, 80, 81
 Opsonic theory of immunity, 24-29

- Opsonin, effect of heat on, 26, 28
 - immune, 26
 - importance in immunity, 25, 97, 107
 - nature of, 28
 - normal, 26, 76
 - percentage index, 81
 - specificity of, 27
 - structure of, 27
- Opsonophore group, 28
- Oral administration of serum, 126, 240
 - of tuberculin, 197

P

- Paracolon and paratyphoid bacilli, 203
- Pathogenesis, 1
- Pertussis, 162-164
 - Bacillus of Bordet and Gengou, 163
 - Differential diagnosis, 163
 - Immunity and specific therapy, 163
- Pfeiffer's phenomenon, 56
- Phagocytic index, 26, 75
- Phagocytic theory of immunity, 11
- Phagocytosis, causes of, 11, 26
- Phagolysis, 11, 161
- Phylacogens, 113
- Plague (*see* Bacillus pestis), 229-230
- Pneumococcus (*see* Micrococcus pneumoniae), 155-161
- Pneumonia (*see* Mic. pneumoniae and Bact. pneumoniae), 155-161
- Pneumonia bacillus, 161-162
- Pneumonia coccus, 155-161
- Polar bodies, 233
- Poliomyelitis, cultivation of causal organism, 90, 261
- Pollantin, 260
- Polyceptor, 21
- Precipitins in diagnosis, 18, 39, 40, 51-55
 - in immunity, 97, 118, 220
- Pure cultures, 36
- Pus, 5, 97

R

- Rabies, 253-259
 - causal organism of, 253
 - Differential diagnosis of, 253-256
 - animal inoculation in, 254
 - Negri bodies, 255
 - Immunity and specific therapy, 256-258
 - Pasteur treatment, 256
 - Prophylaxis, 256, 258
- Reactions from serum injections, 82-85, 126-134, 241
 - from vaccine injections, 82-85, 106
- Reactivation of serum, 119
- Receptors, 13-24
 - of the first order, 17
 - of the second order, 18
 - of the third order, 19
- Rheumatism, acute articular (*see also* Strep. pyogenes), 144, 147, 149
 - gonorrhœal, 151, 152, 154

S

- Salvarsan, 135, 276-280
administration of, 276
chemical constitution of, 277
effects produced by, 279
- Scarlet fever, 80, 143, 146, 149
- Septicæmia, 145-148
- Serum, antibacterial, 19, 118, 120, 124
antitoxic, 117, 120, 124, 221, 235, 244, 260
concentration of, 121-123
cytolytic, 22, 63, 269
disease (*see* anaphylaxis), 83, 126-134, 241
dried diphtheria antitoxin, 240
dried immune, 123, 240, 247, 260
dried tetanus antitoxin, 247
hæmolytic, 22, 63, 269
injection of, intravenous, 126, 237
spinal, 98, 173
subcutaneous, 125
inactivation by heat, 118, 269
local application of, 98, 126, 240, 247
oral administration of, 126, 240
normal serum therapy, 136
purification of, 121-123
reactivation of, 119
standardization of, 120
therapy, 115-137
polyvalent, 147
- Shiga's bacillus (*see* *Bacterium dysenteriae*), 216-225
- Side chain theory of immunity, 13-24
- 606 (*see* salvarsan), 135, 276
- Smallpox, 80-82, 248-253
Causal organism of, 248
Differential diagnosis, 248-250
Immunity and specific therapy, 250-252
Lapine, 251
Vaccination or active immunization, 250
Prophylaxis, 250, 252
- Smears for microscopical diagnosis, 35
for diagnosis of tuberculosis, 176-181
for opsonic index determination, 73, 80, 81
- Spirillum cholerae (*see* *Vibrio cholerae*), 96, 226-228
- Spirochæta microdentium, 266
pallidum, 263, 265, 266
refringens, 266
- Sputum, albumin test with, 88
- Staphylococcus pyogenes (*see* *Micrococcus pyogenes*), 138-143
- Stimulins, 25
- Streptococci, 69, 143
- Streptococcus erysipelatis, 143, 146, 149
- Streptococcus pneumoniae (*see* *Micrococcus pneumoniae*), 155-161
- Streptococcus pyogenes, 91, 143-150
Aronson's serum, 148
Arthritis, 147, 149
Differential diagnosis, 143-144
Erysipelas, 146, 148
Immunity and specific therapy, 144-150
active immunization, 145
passive immunization, 147

- Streptococcus pyogenes*—Cont'd
 serum therapy, 147
 vaccine therapy, 145
 Infections with, 143
 Marmorek's serum, 147
 Menzer's serum, 149
 Moser's serum, 149
 Ruppel's serum, 148
 Scarlet fever, 146, 149
 Septicæmia, 145, 148
 Tavel's serum, 148
Streptococcus rheumaticus, 143, 147, 149
Streptococcus scarletinæ, 143, 146, 149
Sycosis barbæ, 140
Syphilis, 84, 86, 91, 263-280
 Causal organism of, 263
 Chemotherapy, 134, 275-280
 Differential diagnosis of, 263-275
 allergy, 273
 ammonium sulphate test, 273
 Bordet-Gengou reaction, 268
 butyric acid test, 273
 clinical, 264
 cobra venom test, 273
 complement fixation test, 268
 cultural, 267
 epiphanin test, 273
 luetin test, 274
 microscopical, 265
 Noguchi test, 268
 Wassermann test, 268
 Immunity and specific therapy, 275-280
 Neosalvarsan and salvarsan, 276, 277
 Prophylaxis, 280
 Spirochæta pallida, 263-265

T

- Tetanus (*see* *Bacterium tetani*), 242-248
 Toxin, bacterial, 4, 10, 12, 15, 16, 117
 diphtheria, 13, 235
 dysentery, 222
 extracellular, 4, 15, 222, 235, 245
 intracellular, 4, 29
 tetanus, 13, 45
 Toxoid, 16
 Toxophore group, 16
 Tuberculin, Calmette's, 187
 effects produced by, 182-189, 190-198
 in diagnosis, 182-189
 in treatment, 190-198
 kinds of, 191-192
 Tuberculosis (*see* *Bacterium tuberculosis*), 82, 175-200
 Typhoid fever (*see* *Bacillus typhosus*), 84, 91, 204-216
 Typhoid, paratyphoid and paracolon group, 203

U

- Ultramicroscopic organisms, 33, 89-90

V

- Vaccination (*see* active immunization), 100-115
 against smallpox (*see* smallpox), 248-253
Vaccine, autogenous, 104
 bacterial, 103-115
 control of injections of, 107
 dose of, 106
 injection of, 102, 107
 mixed, 99
 preparation of, 103
 stock, 104
Vaccine therapy, 9, 100-115
Vibrio cholerae, 226-228
 Differential diagnosis, 226-227
 Immunity and specific therapy, 227-228
 Infections with, 226
 Prophylaxis, 228
Virulence, 1, 29
Virus, 114
 filterable, 33, 89-90
 vaccine (*see* smallpox), 250-251

W

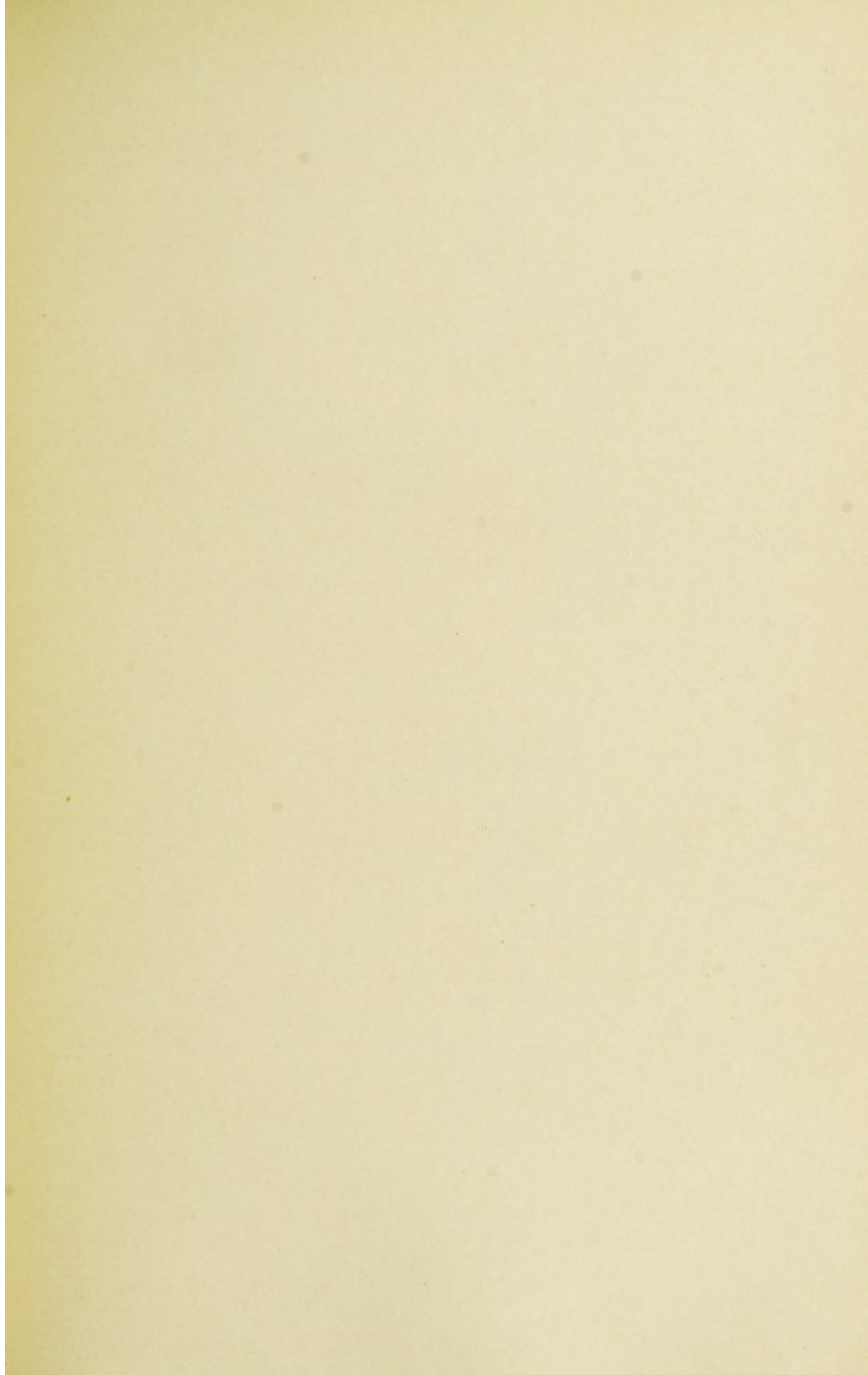
- Wassermann, test in syphilis, 60, 268-273
Whooping cough (*see* pertussis), 162-164
Widal reaction (*see* also agglutination), 45, 207

X

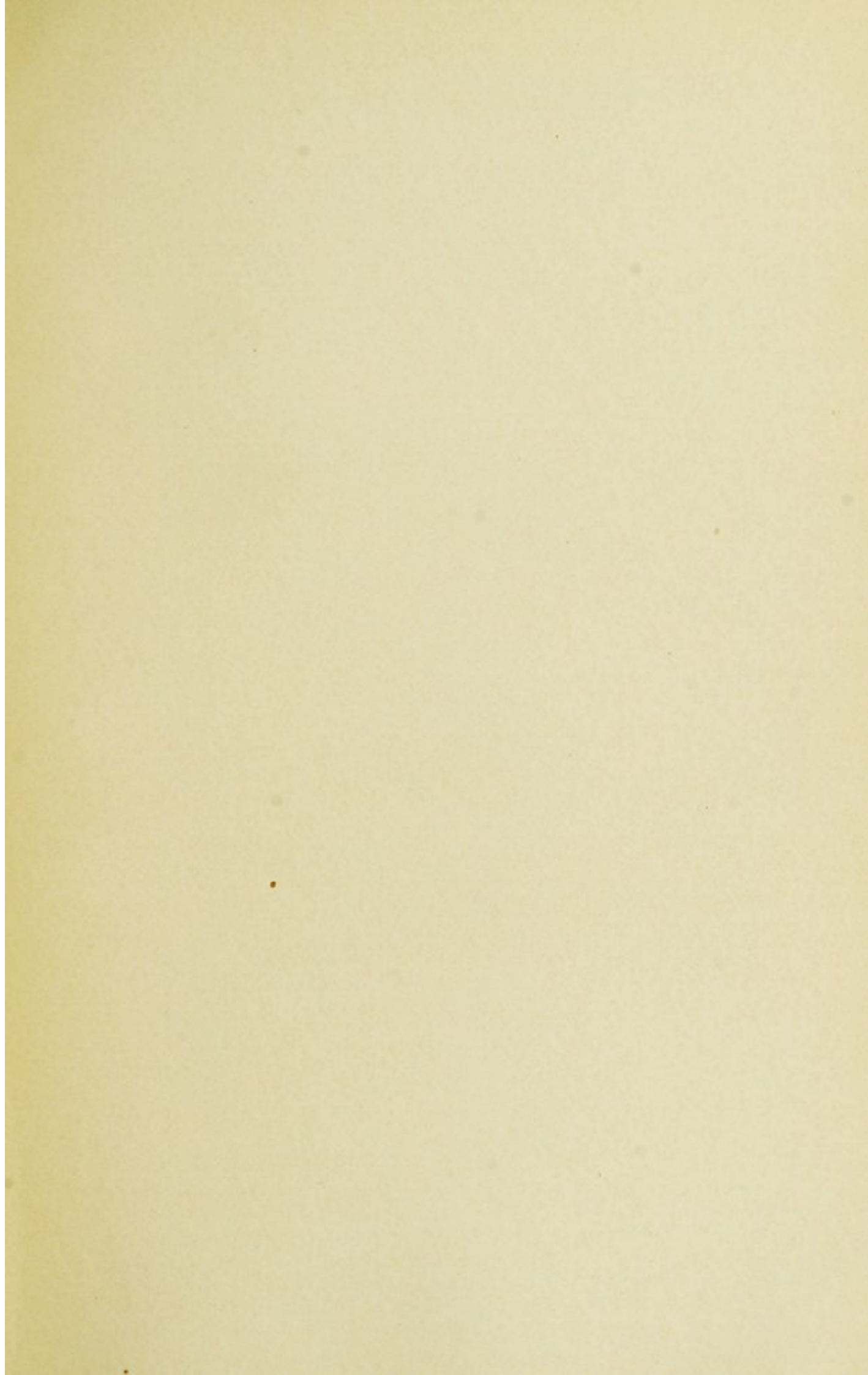
- Xerosis bacillus, 234

Z

- Zymophore group, 20











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